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THE ILLINOIS SPECIES OF MACROSTELES, WITH AN EVOLUTIONARY OUTLINE OF THE GENUS (HEMIPTERA, CICADELLIDAE)

THOMAS E. MOORE AND HERBERT H. ROSS[1]
Illinois Natural History Survey, Urbana

Two circumstances prompted us to undertake this study. First, recent outbreaks of the six-spotted leafhopper in Illinois, especially on cereal and forage crops, and the importance of the leafhopper as a vector of certain plant diseases, indicated the desirability of being able to identify the species with certainty. In the second place, the treatment of the genus by Beirne in 1952 demonstrated the unreliability of many identification characters used in the past for recognition of species in *Macrosteles*. As a result we found it necessary to re-examine all the material of the genus in the Survey collection and to undertake an intensive field program in an effort to obtain host data.

During the course of this study we encountered two species new to science and several new records of the genus for Illinois. To accomodate these we have constructed a new key for the identification of all the Illinois species, which can serve as a substitute for the key published in the "Leafhoppers of Illinois" (DeLong, 1948). A supplemental list of the Illinois species has also been prepared, bringing up to date our records for the genus *Macrosteles* in Illinois.

While attempting to ascertain the relationships of the new species, it was discovered that most of the species of the world fauna of *Macrosteles* are illustrated in sufficient detail to enable us to work out a comprehensive phylogenetic tree for the genus. Although a few phyletic lines could be placed only with some degree of uncertainty, there seems to be little doubt concerning the validity of the major portion of the tree, inasmuch as it brings into harmony evolutionary trends involving several characters. When geographic distribution was plotted on this tree, it was possible to arrive at some interesting conclusions concerning the intercontinental dispersal which has occurred in this genus.

[1]Accepted for publication March 27, 1956.

For information concerning the characters of the North American species we have relied primarily on the paper by Beirne (1952) and for the European species have found the papers by Ribaut (1952) and by Dlabola (1954) extremely useful. We wish to express our indebtedness to these gentlemen in this regard.

Previously established synonymy has been adequately treated in the references cited above and is not repeated here. This includes the acceptance of *Davisonia* and *Sonronius* as subgenera of *Macrosteles* as suggested by Oman (1949), in contrast to the treatment of these groups as genera in the report on Illinois leafhoppers by DeLong (1948).

The scientific names of Illinois plant hosts are given as they appear in Jones (1950).

NOTES ON THE ILLINOIS FAUNA

Thirteen species of this genus, including representatives of all three subgenera, are now known from Illinois. This represents the addition of four species and the subtraction of one from the list in the 1948 Illinois leafhopper bulletin.

KEY TO MALES OF ILLINOIS SPECIES

1. Pygofer bearing an apico-mesal brush of setae (fig. 1) 4
   Pygofer lacking an apico-mesal brush of setae; aedeagal connective attenuate and bearing a strong dorso-basal crest as in figs. 22 & 24 (Subgenus *Davisonia*) 2
2. Aedeagal processes very short, not movably articulated ..... **snowi**
   Aedeagal processes relatively large and articulated as in fig. 2 3
3. Aedeagal processes flared near articulation; angle formed between front margin of aedeagal base and back margin of aedeagal shaft just opposite much less that 15° (usually nearly parallel) ..... **americana**
   Aedeagal processes not flared near articulation; angle mentioned above 30° or greater as in fig. 2 (shaft and base strongly divergent) ..... **major**

4. Pygofer bearing a pair of ventral digitate lobes as in fig. 1 (Subgenus *Macrosteles*) 6
   Pygofer lacking ventral digitate lobes (Subgenus *Sonronius*) 5
5. Aedeagal shaft attenuate and slim, processes narrow and nearly parallel (fig. 3) ..... **arcuata**
   Aedeagal shaft not attenuate and bearing a crest in back, processes stout and strongly incurved as in fig. 4 ..... **clavata**
6. Apex of shaft bearing a pair of small, earlike projections (auricles), and a pair of long processes crossed at their bases with their apices extending ventro-basad in front of the shaft as in figs. 9 & 10 11
   Apex of shaft lacking auricles, but always having a pair of processes which are neither crossed at their bases nor with their apices extending ventro-basad 7
7. Aedeagal processes bifurcate ..... **bifurcata**
   Aedeagal processes not bifurcate 8
8. Aedeagal processes divergent near bases but crossing over each other toward middle (fig. 5) .. **potoria**
   Aedeagal processes divergent throughout, never crossing 9
9. Aedeagal shaft having lateral crenulate flanges which are bent strongly ventro-caudad as in fig. 6; apodemes at base of abdomen as in figs. 13 & 14 ..... **jussiaeae**
   If present, lateral crenulate flanges of shaft not bent ventro-caudad (fig. 7) 10
10. Back of aedeagal shaft bearing a crest (fig. 7); apodemes at base of abdomen as in figs. 15 & 16 ..... **cristata**
    Back of aedeagal shaft lacking a crest; apodemes at base of abdomen as in figs. 17 & 18 .. **fascifrons**
11. Apices of aedeagal processes flared and toothed ..... **lepida**
    Apices of aedeagal processes neither flared nor toothed 12
12. Auricles at apex of shaft extending laterally from near the sides of the gonopore (fig. 9); ventral apodeme at base of abdomen as in fig. 19 ... **variata**
    Auricles at apex of shaft situated on back side of shaft and extending antero-dorso-laterad (fig. 10); ventral apodeme at base of abdomen as in fig. 20 ..... **urticae**

Subgenus **Davisonia** Dorst

Sagatus Ribaut, 1948. Type, by original designation: *Cicada punctifrons* Fallén. *New synonymy.*

The close affinity of *punctifrons* (figs. 23–24), the genotype of *Sagatus*, with *major* and its allies (figs. 21–22), suggests that *punctifrons* may be placed best as a branch of the subgenus *Davisonia*.

Macrosteles americana (Van Duzee)

Nearctic. In Illinois known only from localities near or on rivers from three northernmost counties and generally throughout eastern half of state. The record for Waukegan in the Illinois report is in error, based on a female of *M. snowi*. Recorded host *Salix*. Ribaut (1952, p. 61) apparently was in error in placing this species as a synonym of *punctifrons* Fallén.

Macrosteles snowi (Dorst)

Nearctic. Additional state record: Waukegan, August 24, 1917, 1 ♀ (incorrectly listed in the Illinois report as *delongi*). Recorded host *Salix*.

Macrosteles major (Dorst)

Nearctic. No new records at this time. Recorded host *Salix*; all Illinois localities are on or near rivers.

Subgenus **Macrosteles** Fieber

Erotettix Haupt, 1929 (*fide* Ribaut, 1948: 57). Type, by original designation: *Thamnotettix cyane* Boh. *New synonymy.*

The various species in this subgenus are seen to form a well-marked series of evolutionary changes leading from the more primitive members such as *fascifrons* to the more specialized such as *variata*. The genotype of *Erotettix* fits well into this series of species groups, indicating that it belongs within the subgenus *Macrosteles*.

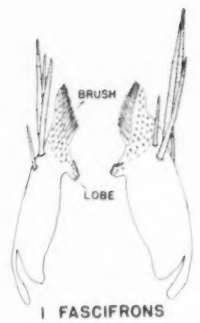
Since completion of the original draft of this paper, Dr. D. A. Young kindly loaned us some Japanese and Korean material of this subgenus containing two species concerning which we had no previous information. *Macrosteles masatonis* Matsumura is a member of the *slossoni-potoria* complex, and *artemisiae* Mats. is a close relative of *lepida*. Both of Matsumura's species fit well into the phylogenetic and dispersal concepts proposed later in this paper, although neither appears on the tree. We could find no validation for the name *traoti* Mats., but the specimens so labeled in Dr. Young's lot were conspecific with those labeled *masatonis*. Specimens of both species bore Dr. Matsumura's determination labels.

It appears to us that an analysis of more characters is needed to establish conclusively the validity of *skalkahiensis* Beirne, 1952, as a species distinct from the very closely related *M. forficula* (Ribaut, 1927). Added to this complex is a form from Japan which closely resembles available figures of *forficula*, as far as aedeagal characters are concerned, but differs from the typical "six-spot" form of both *forficula* and *skalkahiensis* in that it is a secondary "four-spot". We have referred this Japanese form to *forficula*, a species previously known only from France, as a temporary measure until authentic specimens of both species and a more complete record of available names proposed by Japanese authors are available.

There are two species of subgenus *Macrosteles* which we suspect may be found in Illinois at some time in the future, based on the proximity to the state of their present known ranges and the similarity of their distributional patterns to those of some elements of the Illinois fauna. These

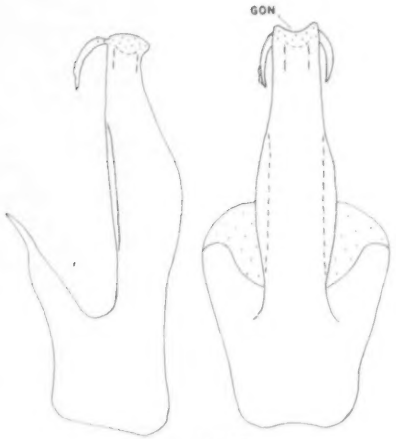
EXPLANATION OF FIGURES

FIGS. 1–10. Genital structures of *Macrosteles* species as indicated. Abbreviations: *GON*, gonopore; *AUR*, auricle. 1, pygofer, ventral aspect; 2, 4–10, aedeagi, left lateral and ventral aspects; 3, apex of aedeagus, ventral aspect.

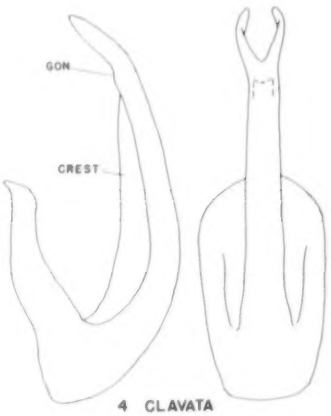


1 FASCIFRONS

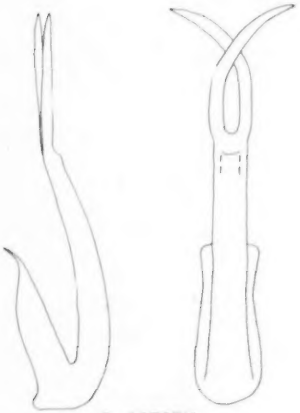
3 ARCUATA



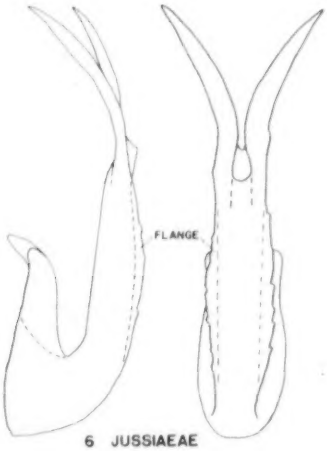
2 MAJOR



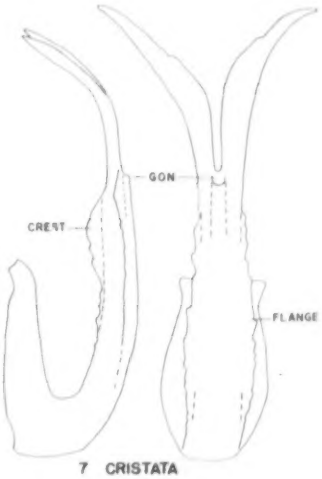
4 CLAVATA



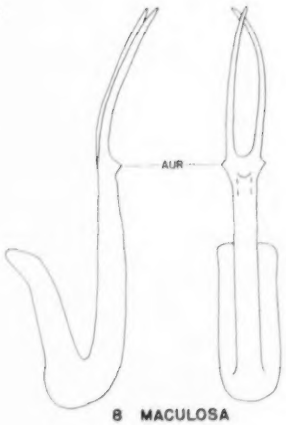
5 POTORIA



6 JUSSIAEAE



7 CRISTATA



8 MACULOSA

9 VARIATA

10 URTICAE

species are *M. divisa* and *slossoni*, for which at present no definite host associations are known. The following annotated list presents the ranges and available plant host information for the species of this subgenus now known from Illinois.

Macrosteles bifurcata Beirne

Nearctic. Originally recorded from Algonquin, Illinois, by Beirne (1952). Additional specimens in the Survey collection, included in the Illinois report with the material of *divisa* (*sexnotata*), are as follows: Algonquin, Illinois, October 5–16, 1895, W. A. Nason, 5♂.

Macrosteles cristata (Ribaut)

Holarctic. First recorded from Algonquin, Illinois by Beirne (1952). Additional records from Illinois are listed below. Some of this material was recorded in the Illinois report as part of the *divisa* (*sexnotata*) series.

In Illinois *cristata* is known from four northern counties and from three other counties about two-thirds of the way down the state, with a considerable hiatus between. Records are as follows: Fulton, July 8, 1934, DeLong & Ross, low pasture, 1♂; Apple River Canyon State Park, July 11, 1934, Frison & DeLong, 1♂; Howard, July 17, 1936, Mohr, 1♂; Algonquin, August 1, 1895, 1♂, May 21, 1896, 1♂; Marengo, Kishawaukee R., July 13, 1955, Sanderson & Evers, 1♂, 4♀; Lake Villa, August 31, 1955, Stannard & Moore, on grasses, 1♂; Alton, June 27, 1934, DeLong & Ross, 1♂; DuBois, June 22, 1905, in field, 1♂; Mt. Carmel, July 3, 1906, 2♂.

No host is known for this species, which is apparently associated with grassy areas along streams and near lakes. It has been recorded from grain and clover as well as grassy places in Europe.

Macrosteles fascifrons (Stål)

Nearactic. First recorded from Illinois by Beirne (1952). Recorded in the Illinois report as *divisa* (*sexnotata*). As pointed out by Beirne, the forms currently assigned to this specific epithet form a species complex exhibiting great geographical and individual variation in both structure and coloration. Found throughout Illinois, and by far the most abundant and widespread member of the genus in both Illinois and North America. Illinois collection dates range from March 27 to October 5.

In Illinois this species has been of economic importance on wheat, oats, carrots, lettuce, and horseradish. Early records (Forbes, 1885) indicate that it was formerly much more abundant on wheat than on oats, but our 1952–1954 collection records indicate a definite shift in preference to oats. Wheat and oats appear to be the only plants on which *fascifrons* causes appreciable damage solely by feeding. On the other crops on which this species has been economically important in Illinois, it has been a vector or suspected vector of plant diseases. Additional hosts from our records are bluegrass, artichoke, carrot, peas, red and sweet clovers, alfalfa, field corn, primrose-willow (*Jussiaea diffusa*), *Polygonum* sp., great nettle (*Urtica dioica*), clearweed (*Pilea pumila*), and wood nettle (*Laportea canadensis*).

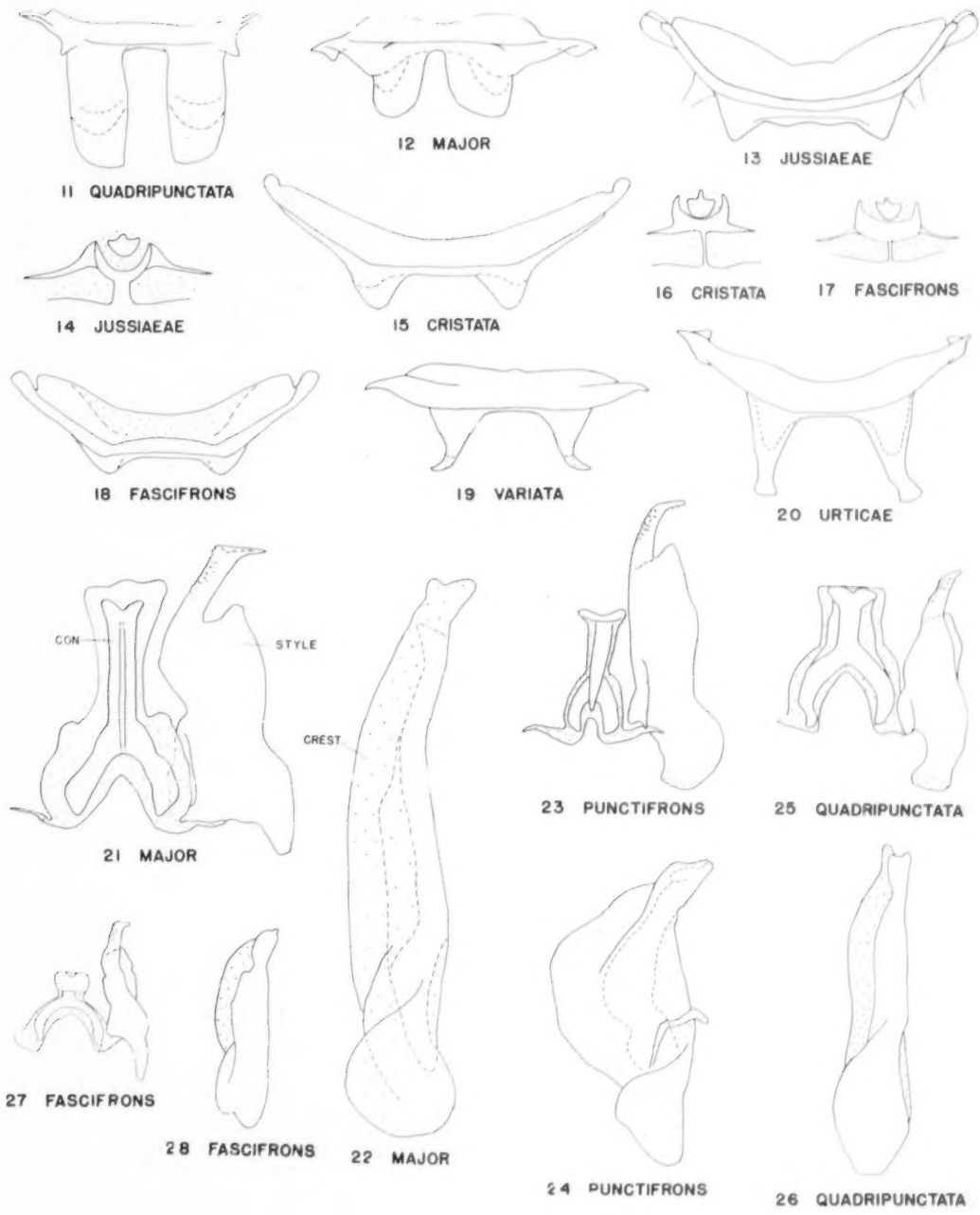
A critical examination of the genitalia of the lectotype male of *M. quadrilineatus* (Forbes) upholds the conclusion of Beirne (1952) that Forbes' species is a junior synonym of *fascifrons* (Stål).

This species is remarkably similar to the European *alpina* (Zett.). In *fascifrons* the apical processes of the aedeagus curve outward for their entire length; in *alpina* these processes curve chiefly outward, but their apexes tend to curve back slightly mesad. These species may perhaps be more readily separated on the basis of characters of the second abdominal sterna as shown in fig. 18 and in Ossiannilsson (1951, fig. 2). The great morphological similarity between *fascifrons* and *alpina* indicates that both species evolved from a common ancestor relatively recently. It is likely that this ancestor was a Holarctic Pleistocene form which became divided into two populations, one in Europe and one in North America. Evolutionary divergence of the two populations since the time of their isolation seems to have been primarily physiological. *Macrosteles alpina* is a species of narrow ecological tolerance, restricted to a few alpine and northern areas, whereas *fascifrons* has evolved a wide ecological tolerance, as shown by its varied list of hosts and its wide geographic distribution. The latter species also is the only known migratory species in the genus *Macrosteles*.

A very interesting series of illustrations of variation in color pattern and in structure of the aedeagus for *M. fascifrons* is given by Beirne (1952). Because of his ample treatment, and the accuracy of the aedeagal illustrations (figs. 481A, B, p. 342) in the Illinois report, no drawings of the aedeagus are given here. It should be noted that the aedeagal figures given for *M. slossoni* in the Illinois report are actually of *fascifrons* (figs. 478 A, B, p. 342).

Macrosteles jussiaeae new species

Male.—Length 3.5–3.7 mm.; head, pronotum, and scutellum yellow-green, head marked with dark brown or black as in typical (six-spotted) *fascifrons*; hemelytra hyaline, infuscated with brown toward apex. General structure of genitalia typical for subgenus. Aedeagus with lateral crenulate ridges strongly bent ventro-caudad (fig. 6). Apodemes of base of abdomen distinctive as illustrated (figs. 13 & 14).



FIGS. 11-28. Abdominal structures and appendages of *Macrosteles* species as indicated. Abbreviation: *CON*, aedeagal connective. 11-13, 15, 18-20, apodemes of second abdominal sterna, ventral aspect *in situ*; 14, 16, 17, dorsal apodeme of base of abdomen, dorsal aspect *in situ*; 21, 23, 25, 27, aedeagal connectives and styles, dorsal aspect; 22, 24, 26, 28, aedeagal connectives, left lateral aspect.

Female.—Length 4.0–4.3 mm.; coloration similar to male; seventh sternite very similar to that of *cristata*.

Holotype, male.—Oakwood, Vermilion Co., Illinois, July 12, 1955, Moore & Kingsolver, on *Jussiaea diffusa*.

Allotype, female.—Marion, Illinois, July 7, 1955, Ross & Moore, on *Jussiaea diffusa*.

Paratypes, Illinois.—Same data as for holotype, 66♂; Oakwood, July 12, Moore & Kingsolver, on *Nymphaea, Potamogeton*, and *Jussiaea*, 1♂; Salem, July 8, 1955, Ross & Moore, on *Jussiaea diffusa*, 16♂, 4♀; Gorham, July 8, 1955, Ross & Moore, on *Jussiaea diffusa*, 1♂, 2♀; Carlyle, July 8, 1955, Ross & Moore, on *Jussiaea diffusa*, 7♂, 4♀; Marion, July 7, 1955, Ross & Moore, on *Jussiaea diffusa*, 2♂, 2♀; Woodbury Lake, Woodbury, August 23, 1955, Ross & Selander, on *Jussiaea diffusa*, 30♂, 5♀; Grand Marais St. Park, September 28, 1955, Selender & Moore, on *Jussiaea diffusa*, many ♂ and ♀.

Host.—*Jussiaea diffusa* Forsk (primrose-willow).

This species appears to be most closely related to *M. cristata*. Males can be separated on the basis of characters of the aedeagus and abdominal apodemes. The observed variation in aedeagal structures ranges from bending forward of the processes in one male from Woodbury Lake, Woodbury, Illinois, to suppression of the crest on the back of the aedeagal shaft.

M. jussiaeae has been collected in the southern third of the state, with an isolated northward extension in Vermilion Co. If this species follows closely the range of its host, as it appears to do, we would expect to find an additional isolated northward extension in Mason Co.

Macrosteles lepida (Van Duzee)

Nearctic. Recorded correctly in the Illinois report, except that the records for Champaign, Dolson, Dubois, and Havana were in error. The latest collection date is October 8 instead of August 25. Additional Illinois localities as follows: Fox Lake, Apple River Canyon State Park, White Heath, Temple Hill, Antioch, Wilmington, Beach, and Meredosia. No host known.

Some of our Illinois specimens have a very small auricle near the base of each aedeagal process, similar to the condition in *variata*. This characteristic is important in placing this species on the *variata* line (fig. 34), rather than as a separate line paralleling the four-spot condition of the *variata* group.

Macrosteles potoria Ball

Nearctic. Recorded in the Illinois report as *potoria* and as *slossoni*, the four females of the latter species all properly belonging in *potoria*. Additional Illinois localities: McHenry and Meredosia. Known in this state from the northeastern corner and from along the Illinois River. Only host recorded is *Eleocharis* (spike rush). The figure of genitalia for *slossoni* on page 342 of the Illinois report pertains to *fascifrons* as pointed out above. The former species is not known from Illinois.

Macrosteles urticae new species

Male.—Length 3.6–3.8 mm.; head, pronotum, and scutellum yellow-green; dark markings of dorsum of head supressed except for an anterior and a posterior pair of large spots; dark markings of scutellum prolonged longitudinally in a pair of lobes visible beyond pronotum, often with a transverse linear dark mark behind these lobes; hemelytra somewhat uniformly fuscous, or hyaline and variously marked with brown, often in such a manner as to delimit a hyaline saddle in the region of the apices of the anal veins. Genitalia with general structure typical for subgenus. Auricles or ears of aedeagus on back side of shaft and extending antero-dorso-laterad (fig. 10). Ventral apodemes at base of abdomen as illustrated (fig. 20).

Female.—Length 4.0–4.6 mm.; coloration similar to male; seventh sternite of abdomen very similar to that shown for *variata* by Beirne in fig. 16.

Holotype, male.—Carthage, Hancock Co., Illinois, September 20, 1955, Ross & Moore, on *Urtica dioica* and *Pilea pumila*.

Allotype, female.—Same data as for holotype.

Paratypes, Illinois.—Same data for holotype, 2♂, 4♀, 3 nymphs; Bureau, September 16, 1955, Ross & Stannard, on *Laportea canadensis*, many ♂ and ♀; Elizabeth, September 21, 1955, Ross & Moore, on *Laportea canadensis* and *Pilea pumila*, 2♂, 2♀, June 29, 1935, DeLong & Ross, 6♂; Volo, July 27, 1934, DeLong & Ross, 26♂, August 24, 1935, DeLong & Ross, in bog, 1♂.

Hosts.—*Urtica dioica* L. (great nettle), *Pilea pumila* (L.) A. Gray (clearweed), and *Laportea canadensis* (L.) Gaud. (wood nettle).

Macrosteles urticae is probably most closely related to the *variata* complex, and may be distinguished on the basis of the characters illustrated. There is some variation in the relative size of auricles on the aedeagus. Most auricles observed are similar in size to those illustrated. Inclination of aedeagal processes also varies, with the inclinations illustrated indicating the approximate extremes. In most cases the two processes are inclined to about the same degree.

This species is known from the northern half of Illinois. If *Urtica dioica* is the critical host, then one would expect to find *Macrosteles urticae* only in this area. However, we do not know that such is the case and the other two known host plants are more or less statewide in distribution, suggesting the need for a good deal more

collecting on urticaceous plants in the southern part of the state.

Macrosteles variata (Fallén)

Holarctic. This is another species complex, similar in many aspects of variation to *M. fascifrons*. We are able to validate only seven of the records given in the Illinois report. The collection dates of these records range from May 23 to August 8: DuBois, Elizabeth, Fern Cliff, Metropolis, Mounds, Shawneetown, and White Heath. Known from the southern third and northwest corner of the state, and from one central county.

Recorded hosts: *Impatiens* (jewel-weed), *Urtica dioica* (great nettle), sugar beet, and carrot.

Subgenus **Sonronius** Dorst

Macrosteles arcuata (Gillette & Baker)

Nearctic. The Illinois report listed this species as *binotata*. The name *binotatus* Sahlberg, *sensu* Dorst (1937), however, was based on a misidentification of *arcuata*. In Illinois known from Waukegan, Lake County. No previously recorded hosts. Specimens at hand from British Columbia were collected on potato and in alpine meadows. The illustration of the tip of the aedeagus of this species in Beirne (1952) is in error (*cf.* fig. 3). Ribaut (1952, p. 60) apparently was incorrect in placing this species as a synonym of *binotatus* Sahlberg.

Macrosteles clavata (Delong & Davidson)

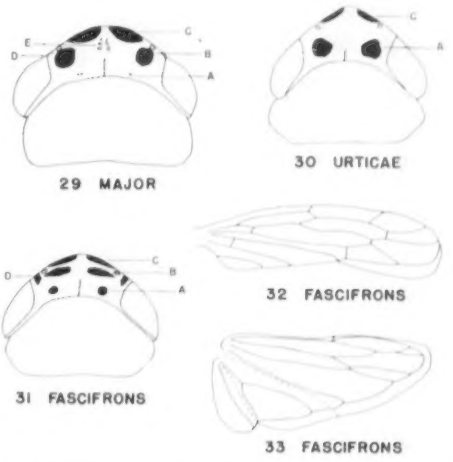
Nearctic. Recorded in the Illinois report as *Sonronius clavatus*. Known only from a single specimen from Lake County in Illinois. No host known.

EVOLUTIONARY DEVELOPMENT

About fifty valid species have been described in the genus *Macrosteles*. Of these, 47 are sufficiently well known and illustrated to be included in this phylogenetic treatment. In addition to those species which occur in Illinois, we have examined specimens of the following species of *Macrosteles: artemisiae, borealis, divisa, elongata, forficula, laevis, masatonis, osborni, punctifrons, quadripunctata, sexnotata,* and *slossoni*. Specimens of many of these species were obtained through the kindness of Dr. B. P. Beirne, Canadian Department of Agriculture, and Dr. D. A. Young, U. S. Department of Agriculture.

In surveying the characters of species of *Macrosteles* from various parts of the world, certain evolutionary trends can be seen. We have tabulated characters of head color and male genitalia for the 47 species of which specimens or adequate illustrations are available, and almost all can be placed with considerable reliance along the lines of these trends (see fig. 34). A few species are difficult to place and appear to represent side trends for which little evidence of their relationships is available.

The most conspicuous basis for grouping the species is in the structure of the pygofer. In the subgenus *Davisonia* the pygofer is simple, *i.e.* lacks both the brush of setae and digitate lobe shown in fig. 1. In the subgenus *Sonronius* the pygofer has a series of short stout setae forming a brush along the apico-mesal margin (see fig. 1). In the subgenus *Macrosteles* the pygofer has the brush as found in *Sonronius* and in addition a ventral digitate lobe (fig. 1).



FIGS. 29-31. Dorsal aspect of head and pronotum of *Macrosteles* species as indicated. FIGS. 32, 33, dorsal aspects of right hemelytron and hind wing respectively.

A striking trend can be seen in the color pattern of the head. It would appear that the ancestral number of head spots in this group is five pairs (one spot for each pair is labeled in fig. 29 as *A*, *B*, *C*, *D*, and *E*). From this ancestral type three general specializations were developed. In *Davisonia* (fig. 29) all but the large *B* and *C* spots are either completely suppressed, or only vaguely indicated. In *Sonronius* usually all but the *B* spots are suppressed, although some individuals have one or two other pairs of spots present. A notable exception to this rule in *Sonronius* is *quadripunctata*, which always has the *C* spots, and sometimes has all five pairs of spots definitely present. In most species of subgenus *Macrosteles* (fig. 31) spots *A*, *B*, *C*, and *D* are definitely present and fairly large, the *B* spots having been elongated into bars (the typical "six-spot" condition). Possibly the elongated *B* spots represent a fusion of the ancestral *B* and *E* spots. In two independent phyletic lines of subgenus *Macrosteles* (the *quadripunctulata* and *variata* groups) the *A* spots have become quite large and the *B* spots entirely

suppressed or only vaguely indicated (fig. 30). This forms a "four-spot" condition superficially resembling that of *Davisonia* (fig. 29). Individual variation in certain species of subgenus *Macrosteles* ranges from complete suppression of some or all pairs of spots to complete melanism of the dorsum of the head. For examples of this latter condition see Beirne (1952).

We can also recognize a general evolutionary tendency in characters of the styles and aedeagal connective. In *Davisonia* (figs. 22 and 24) and in *Sonronius* (fig. 26) the connective is relatively elongate and bears a prominent dorsal crest. But in the subgenus *Macrosteles* the connective is noticeably shortened and has only a very short low crest (fig. 28). The form of *Sonronius* is somewhat intermediate between these two extremes. The styles of each group have a more or less distinctive configuration as illustrated (figs. 21, 23, 25, and 27).

Several trends of structural modification of the aedeagus can be observed in this genus. The primitive condition probably was characterized by a simple terete, tubular aedeagal shaft with an apical gonopore and with an apical pair of slightly back-curving, simple processes. In the *Davisonia* group, as depicted for ***major*** (fig. 2), the general tendency is for further bending backwards, with the connection between the shaft and processes becoming membranous and the processes becoming articulated. In the species of *Sonronius*, represented by *clavata* (fig. 4), the processes are short and the gonopore is located either apically or slightly subapically on the dorso-cephalic side. A further variation among some of these species is the occurrence of a crest on the back of the aedeagal shaft.

The primary feature of the aedeagus in the subgenus *Macrosteles* is that the gonopore is subapical on the ventro-caudal side as shown for *jussiaeae* and the remaining species (figs. 6–10). Evolution within the subgenus is well illustrated by characters of the apical processes of the aedeagus. In the first two phyletic lines, as illustrated for *cristata* and *jussiaeae*, the processes are simple and back-curved (figs. 6 and 7),

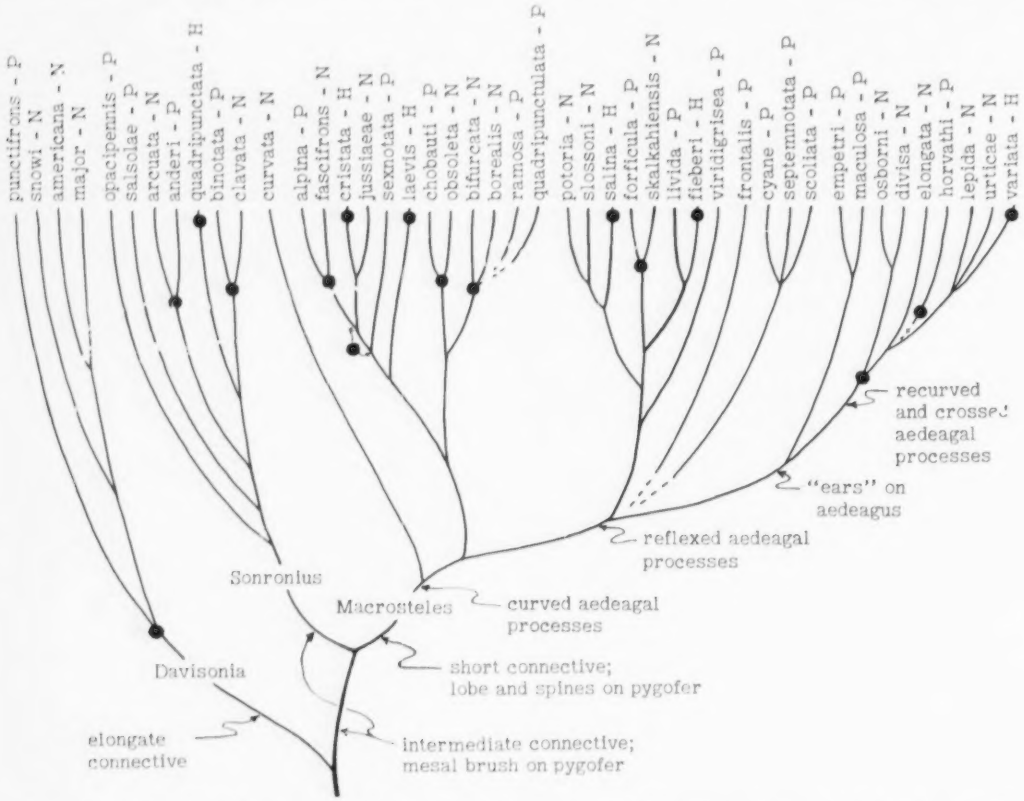


FIG. 34. Chart of phylogeny and distribution of *Macrosteles*. The dark circles indicate the postulated occurrences of Holarctic dispersals. Abbreviations: *H*, Holarctic Region; *N*, Nearctic Region; *P*, Palearctic Region.

except for their becoming bifurcate in the *chobauti-quadripunctulata* series. Before the line giving rise to *potoria* (fig. 5) diverged, the processes appear to have become reflexed, with the result that their apices now point in a ventro-caudal direction. Following this a pair of auricles developed on the aedeagal shaft near the bases of the processes as seen in the figure for *maculosa* (fig. 8). Finally specialization on this line to the *variata* type (see figs. 9 and 10) took place, in which the processes became crossed at their bases and so strongly recurved that their apices extend in a ventro-cephalic direction.

On the basis of these trends, it seems reasonable to conclude that the genus *Macrosteles* followed the evolutionary development depicted in figure 34 and outlined below. The genus arose from an ancestral form combining the simple pygofer of *Davisonia* with five pairs of head spots as found in some individuals of *Sonronius quadripunctata*. The gonopore was at the apex of the aedeagal shaft. It is likely that the connective was of the intermediate type exemplified by *Sonronius*. This ancestor gave rise to a branch represented today by the subgenus *Davisonia* and a second represented by the subgenera *Sonronius* and *Macrosteles*. In the *Davisonia* branch the number of head spots was reduced, those spots remaining were quite enlarged (fig. 29), and the connective became elongate and crested (figs. 22, 24). Otherwise the line changed little. In the other branch the pygofer evolved its apical brush (fig. 1). This line early gave rise to *Sonronius*, which specialized in general in the suppression of certain head spots (similar to the condition in *Davisonia*) and the movement of the gonopore to the dorso-cephalic side ofthe aedeagus, and the *Macrosteles* line, in which the pygofer developed its ventral lobe, the gonopore became caudal and subapical in position, the connective was greatly shortened, and the six-spot condition of the head shown in fig. 31 became fixed. Evolution within the subgenus *Macrosteles* followed the outline described above in explaining the trends shown by the apical processes of the aedeagus and by head coloration.

HOST RELATIONSHIPS

Host preferences are known for about 24 species. All four species of *Davisonia* are recorded from *Salix*. In *Sonronius* hosts are recorded for four species: *salsolae* on *Salsola*, *arcuata* on *Solanum*, *quadripunctata* on *Epilobium* and *Rubus*, and *binotata* on Salicaceae.

In the subgenus *Macrosteles* the species *cristata* and *sexnotata* are recorded from grasses; *fascifrons* feeds on grasses and many forbs such as *Daucus*, *Jussiaea*, and *Urtica; jussiaeae*, a close relative, seems to feed only on *Jussiaea*. In other complexes of *Macrosteles* an equally hetergeneous list of host preferences has been recorded, but the end branch contains three species (*elongata*, *urticae*, and *variata*) which feed only on *Urtica* or its allies.

From these data no definite evolution of host trends can be deduced. Possibly grasses were the ancestral host of the subgenus *Macrosteles*. If so, occasional species lines have switched to other hosts in a random fashion.

DISPERSAL

When general areas of distribution (Nearctic, Palaearctic, Holarctic) are plotted for each species on the family tree it is evident that few species groups developed within the confines of a single geographic area, but rather that most lines have spread from continent to continent. This results in a dispersal pattern for the whole group which is extremely complicated when compared with those of groups such as the tribe Agapetini in the Trichoptera which have dispersed between continents very little (Ross, 1953). In attempting to unravel the *Macrosteles* dispersal pattern we have determined the minimum possible number of Holarctic dispersals necessary to account for present-day distribution, each dispersal being indicated by a black spot in fig. 34. Although it is possible that even more Holarctic dispersals actually took place, there is no need to postulate additional dispersals in interpreting existing data. The minimum number of dispersals required is 16.

We have not indicated geologic age in the chart in fig. 34. In the case of living species of Holartic distribution, it is reasonable to suppose that each evolved in either Eurasia or North America and has spread into the other continent during relatively recent time, most likely during the last ice age. Otherwise, based upon what has happened in other branches of the group, populations of these Holarctic species on the different continents would have evolved into discreet entities by this time. Another group of dispersals share the common characteristic that each has given rise to species still extremely similar in morphological characters. These dispersals include the ancestral forms of *arcuata-anderi*, *alpina-fascifrons*, *chobaulti-obsoleta*, and *forficula-skalkahiensis*. The uniformly small amount of change which has occurred within each of these branchlets is suggestive of a simultaneous and moderately recent dispersal of their ancestral forms. This postulated event may have occurred in pre-Wisconsin Pleistocene. There seems to be no indication of the time of the older dispersals. They could have occurred earlier in the Pleistocene, in the Pliocene, or even earlier.

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NEW SPECIES IN THE EMPOASCA FABAE COMPLEX (Hemiptera, Cicadellidae)[1]

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The source of spring populations of the potato leafhopper, *Empoasca fabae* (Harris), in the eastern and midwestern United States has invited much speculation during recent years. Until our present studies, the species was considered to be extremely wide-ranging, with abundant records supposedly of this species from Canada to Argentina. As a result of this concept, the possibility appeared to exist that Caribbean, Central American, or even South American winter populations of *Empoasca fabae* might serve as a source of spring wind-borne migrations into the United States. The thought occurred to us that, if *fabae* had such a large range, it might exhibit local or subspecific morphological types which would "label" suspected spring migrants as to their general area of origin. In an effort to obtain clues bearing on this problem, we embarked on a study of large samples of *fabae* from various tropical localities.

We wish to express our gratitude to cooperating entomologists in the North Central region for collecting much *Empoasca* material, especially within continental U. S. A. In addition, large samples, often numbering a thousand specimens, were received from the following countries and persons: Colombia (R. F. Ruppel); Cuba (H. B. Mills, Valdez Barry); Haiti (H. B. Mills); Honduras (E. C. Becker, H. D. Koone); Mexico (J. H. Lilly, W. W. Gibson, R. B. Selander); Nicaragua (Paul Kraemer); Panama (R. M. Altman, Elliot Williams, W. H. Wymer); Puerto Rico (Irving Fox); United States (F. F. Bibby, J. E. Porter, L. D. Newsom, and many others).

When this material was sorted to fine morphological units, it was apparent that forms keying to *fabae* in existing literature represented several distinct species. Some collections contained only one species, whereas other collections (especially from light trap catches) contained mixtures of several. To date eight species units of the *fabae* complex have been differentiated, diagnosed by characters of the male abdomen.

A NEW CHARACTER

Scrutiny of the base of the male abdomen revealed a set of apodemes arising internally from the juncture of the second and third tergites. In most species of *Empoasca* these apodemes are little more than inconspicuous thickenings along the suture, but in two new species of the *fabae* complex, *hastosa* and *eccla*, the apodemes project posteriorly as sclerotized crescents, figs. 1, 2. A rapid survey of other species shows that here and there throughout the genus these same structures occur. They are as constant in form and in occurrence within a species as are the sternal apodemes.

THE *fabae* COMPLEX

The following combination of characters applies to all the species described below and defines the *fabae* complex: length varying from 3.5 to 4 mm.; color in life pale green with whitish markings on head and thorax; male genitalia with simple aedeagus as illustrated by DeLong (1931), hook of tenth segment with enlarged base and slender sclerotized ventral point, style with only a few short lateral setae and with tip oblique, and

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[2]With the Illinois Natural History Survey at the time the studies reported in this paper were initiated.

brachone having a widened basal portion, a tapering apical portion, and a mesal membranous area occupying the concavity beyond the thicker part of the brachone. To date no characters are known for identifying females to species.

A word of warning is appropriate at this point. In specimens which have been parasitized by Dryinidae, the genital structures and the apodemes at the base of the abdomen fail to develop fully and may be entirely different from the shape found in normal members of the species. It is often not easy to recognize dryinized specimens, but they have weakly sclerotized and reduced parts, have unusually thin and weak abdominal walls, and in KOH produce flabby preparations which appear similar to the teneral condition. Dryinized specimens are most abundant in samples gathered directly from host plants; few are encountered in material collected at light.

Types erected in this paper are in the Illinois Natural History Survey; representative paratypes will be deposited in the U. S. National Museum and the D. M. DeLong Collection.

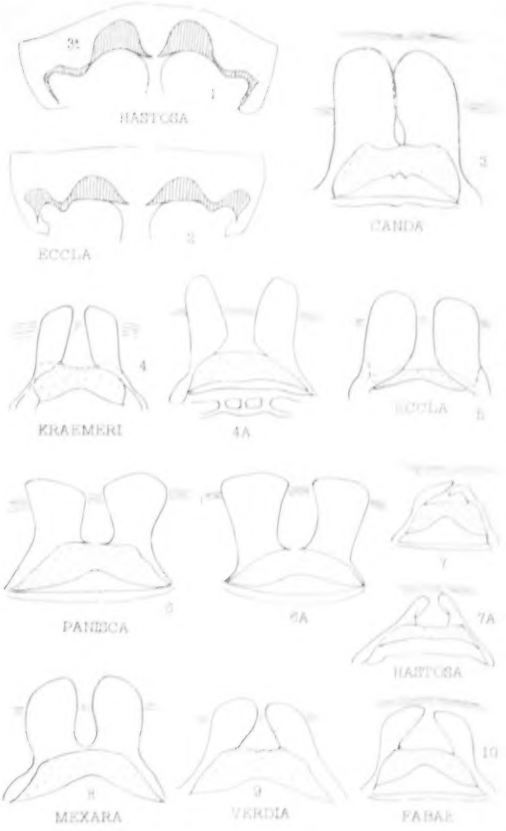
Empoasca fabae (Harris)

Male.—Hook of tenth segment with apical point fairly short, the ventral margin of the base sloping obliquely into the point (fig. 11*D*); styles only moderately sinuate (fig. 11*F*); brachone (fig. 11*A*, *B*) with apical portion curved mesad, the extreme tip sometimes thicker than here illustrated. Tergal apodemes linear, inconspicuous. Sternal apodemes short, narrow and widely separated at base, the mesal margins extremely thin and difficult to identify, the pair of apodemes therefore usually appearing as a single short U-shaped structure (fig. 10).

The name *fabae* has been associated arbitrarily with the form so determined by DeLong (1931) and Medler (1942), and listed by them as occurring throughout central and eastern U. S. Since much North American synonymy is involved and many of the old types are lost, this application of the name seems to be the best procedure at the present time. Names based on types from other countries, such as *Typhlocyba pholophila* Berg described from Argentina, must be considered as unidentifiable pending study of types, if available, rather than as synonyms of *fabae*. In material which we have personally examined to date, typical *fabae* has not been encountered outside territorial U. S. A.

Empoasca hastosa n. sp.

Male.—Size, color, and general structure typical for *fabae* complex. Base of abdomen with well developed tergal apodemes (fig. 1), in which the mesal lobes are large but the lateral portions are slender. Sternal apodemes (fig. 7, 7*A*) reduced to a pair of small finger-like processes well separated at the base but converging rapidly and sometimes overlapping at the apex. Hook of tenth segment (fig. 15*D*) with a long and very slightly sinuate ventral spine, the bulbous portion of the hook narrowing abruptly where it joins the spine. Brachone (fig. 15*A*) with wide basal portion short, narrowed apical portion unusually long, the extreme and slightly widened tip above the end of the membrane also unusually long. Styles sinuate as in fig. 14*G*.



FIGS. 1–10. Apodemes at base of abdomen in *Empoasca*. 1–2, tergal apodemes; 3–10, sternal apodemes. 4, specimen from Florida; 4*A*, specimen from Honduras. 3*t*, third tergite. 6, specimen from Cuba; 6*A*, specimen from Honduras. 7, specimen from Honduras; 7*A*, specimen from Florida.

Holotype.—La Ceiba, Honduras, June 17, 1949, at light, E. C. Becker. *Paratypes.*—HONDURAS: Same data as holotype, May 13-June 27, 43 ♂; 12 km. west of Olanchito, at light, E. C. Becker, July 7–29, 1949, 2 ♂, Sept. 8, 1949, 1 ♂. Additional material studied is from Cuba (Soledad), Mexico (Tecpatan, Chiapas), Panama (Canal Zone), and USA (Miami, Florida).

This is a truly distinctive species which represents an extreme in the development of the tergal apodemes and in the reduction of the sternal

apodemes. In addition, the elongate tip of the brachone is distinctive. Judging from the few collections at hand, this is one of the very common species on beans in many areas of the Central American and Caribbean regions.

Empoasca kraemeri n. sp.

Male.—Typical in size, color, and general characteristics for *fabae* complex. Base of abdomen with tergal apodemes undeveloped. Sternal apodemes moderately widely separated at base and extending distinctly beyond the next intersegmental suture; each apodeme somewhat rectangular, the inner margins nearly straight (fig. 4), except when the apodemes are distorted out of position in which case the inner margin may appear convex (fig. 4*A*). Hook of tenth segment (fig. 14*D*) with long and slightly sinuate ventral spine and almost semicircular base. Brachone (fig. 14*A*, *G*) with widened basal portion fairly short, narrowed portion elongate and sinuate, the extreme tip curving slightly laterad. Style moderately sinuate (fig. 14*G*).

Holotype.—Managua, Nicaragua, April 30, 1956, on beans (*Phaseolus vulgaris*), Paul Kraemer. *Paratypes.*—NICARAGUA: Same data as holotype, many males; same but on squash (*Cucurbita pepo*), 3 ♂. Additional material studied is from Colombia (Medellin, on beans), Cuba (Santiago de las Vegas, on beans), Honduras (La Ceiba, Olanchito, and Tegucigalpa), Mexico (Tecpatan, Chiapas), Panama (Canal Zone), Puerto Rico (Isla Verde), and U.S.A. (Miami, Florida).

This species is closest to *canda* and *verdia*, from both of which it may be separated as outlined in the following key. The rectangular apodemes and the elongate and sinuate apexes of the brachones differentiate the species from all others in the complex. Like *hastosa*, it is one of the common bean species, having been taken in great numbers on this host in Mexico, Nicaragua, and Colombia. It is very likely that this is the form which was recorded by Ruppel and DeLong (1956) as *fabae* from Colombia.

Empoasca panisca n. sp.

Male.—Size, color, and general structure typical for *fabae* complex. Base of abdomen with only linear, inconspicuous dorsal apodemes. Sternal apodemes (fig. 6, 6*A*) extending into succeeding segment, each constricted at or before middle and hence somewhat clavate with the apical margin only moderately convex. Hook of tenth segment (fig. 16*D*) with ventral point tapering and sharp. Brachone unusually wide at shoulder (fig. 16*C*); the narrow apex only half the length of the wider base (fig. 16*A*, *B*) the junction of the two abrupt. Styles slightly more sinuate than in fig. 11*F*.

Holotype male.—Soledad, nr. Cienfuegas, Cuba, June 2, 1950, at light, H. B. Mills. *Paratypes.*—CUBA: Same data as holotype, June 1–6, 12 ♂. HONDURAS: La Ceiba, May 28–June 16, 1949, at light, E. C. Becker, 4 ♂; 12 km. west of Olanchito, July 21 and Sept. 7, 1949, at light, E. C. Becker, 2 ♂.

The short apical narrowed portion of the brachone sets off this and the following species, *eccla*, as a pair distinct from all the other species of the *fabae* complex. In *panisca* the lateral aspect of the brachone is wide at shoulder, the tergal apodemes are undeveloped, and the sternal apodemes are subclavate, whereas in *eccla* the lateral aspect of the brachone is narrow at the shoulder, the tergal apodemes are well developed, and the sternal apodemes are ovoid.

Empoasca eccla n. sp.

Male.—Size, color, and general structure typical for *fabae* complex. Base of abdomen with large, crescentic tergal apodemes (fig. 2), each with an enlarged lateral appendix. Sternal apodemes (fig. 5) ovate, moderately short, and the two well-separated at base. Hook of tenth segment (fig. 17*D*, *E*) with ventral point straight and unusually slender. Brachone with short, narrow apical portion, its lateral aspect (fig. 17*C*) narrowing at shoulder, its ventral aspect (fig. 17*A*, *B*) having only a shallow shoulder. Styles slightly more sinuate than in fig. 11*F*.

Holotype male.—Headquarters, 15th Naval District, Canal Zone, Panama, Nov. 30, 1956, at light, W. H. Wymer. *Paratypes.*—PANAMA: Same data as holotype, 2 ♂. HONDURAS: 12 km. west of Olanchito, July 29, 1949, at light, E. C. Becker, 1 ♂.

The relationships and identification characters of this species are treated under the preceding species and in the key.

Empoasca canda n. sp.

Male.—Size, color, and general structure typical for *fabae* complex. Base of abdomen with undeveloped tergal apodemes. Sternal apodemes long (fig. 3), touching or nearly so at base, their mesal margins approximate and parallel, and each apodeme rounded or subtruncate at apex. Hook of tenth segment varying from a condition much as in fig. 11*D* (in the holotype), with the base sloping gradually into the ventral spine, to the condition in fig. 14*D*. Brachone (fig. 13*A*) moderately elongate, the narrow apical portion curving very slightly mesad, with a slight lateral notch just before tip. Styles (fig. 13*F*) moderately sinuate.

Holotype male.—12 km. west of Olanchito, Honduras, July 19, 1949, at light, E. C. Becker. *Paratypes.*—COLOMBIA: Medellin, Ant., Nov. 7, 1956, on beans, R. F. Ruppel, 1 ♂. CUBA: Santiago de las Vegas, Habana, Sept., 1956, on beans, Valdez Barry. HAITI: Port-au-Prince, May 22, 1950, at light, H. B. Mills, 1 ♂.

HONDURAS: Same data as holotype, July 18–21, 3 ♂, Sept. 7, 1 ♂. PANAMA: Headquarters 15th N.D., Canal Zone, Nov. 30, 1956, at light, W. H. Wymer, 2 ♂. PUERTO RICO: Isla Verde, Oct. 21, 1956, at light, I. Fox, 3 ♂.

This species, readily identified by its proportionately longer ventral apodemes, varies considerably in both this characteristic and the spine of the tenth segment. The apodemes range from the shorter type present in the holotype (fig. 3) to a longer condition in which the two apodemes are situated a short distance apart, extend into one more segment, and are almost truncate at the tip. All intergrades occur between these two conditions.

Empoasca mexara n. sp.

Male.—Size, color, and general structure typical for *fabae* complex. Base of abdomen with undeveloped tergal apodemes. Sternal apodemes (fig. 8) moderately short and ovate, their bases wide, touching or nearly so; in some paratypes the main bodies of the apodemes are farther apart than illustrated in fig. 8. Hook of tenth segment as in fig. 11*D* but with the ventral spine a little longer. Brachone as in *fabae* (fig. 11*A*, B), the apex curved mesad. Styles only slightly sinuate.

Holotype male.—Irapuato, Gto., Mexico, July 15, 1948, on alfalfa, Abarca. *Paratypes.*—MEXICO: Same data as holotype, 5 ♂; Atlixco, Puebla, June 16, 1955, R. B. & J. M. Selander, 4 ♂. U.S.A.: Yuma, Arizona, Oct. 2, 1954, on alfalfa, McCain, 1 ♂.

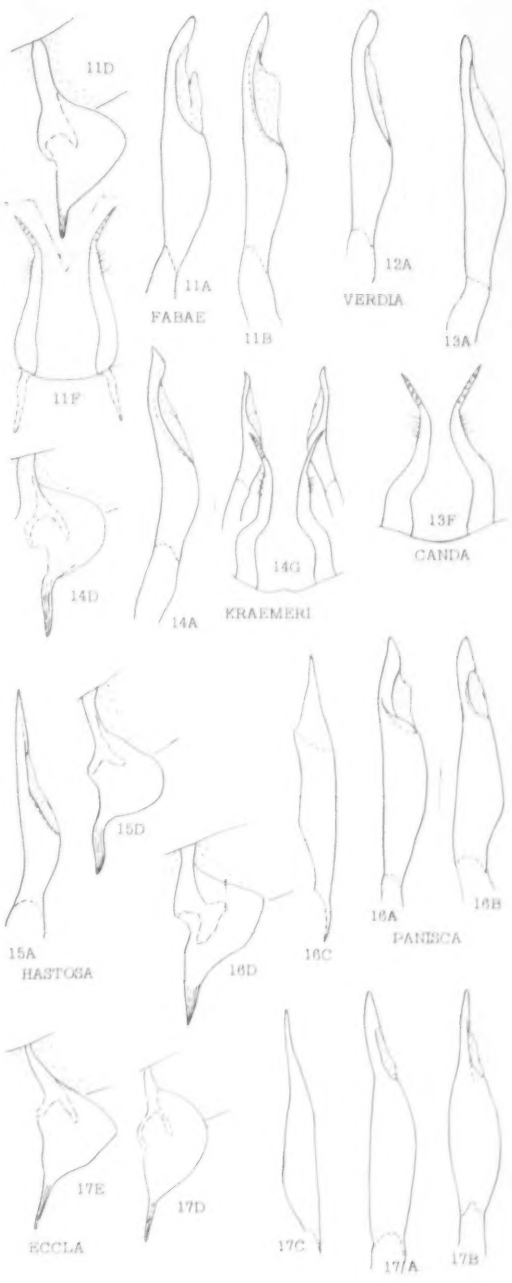
There is a distinct possibility that ultimately this species may prove to be a subspecies of *canda*. At present the two forms, *canda* and *mexara*, appear to be allopatric. They differ chiefly in the length of the sternal apodemes and slightly in the shape of the apex of the brachone. This problem will probably not be settled one way or the other until many more collections have been examined critically from Mexico and countries to the southeast of it.

Empoasca verdia n. sp.

Male.—Size, color, and general structure typical for *fabae* complex. Base of abdomen with undeveloped tergal apodemes. Sternal apodemes (fig. 9) fairly small, far apart at base and converging at apex, each apodeme ovate or with the mesal margin approaching a straight-sided condition, the mesal margin as heavily sclerotized as the lateral margin. Hook, brachone (fig. 12*A*), and styles as in *fabae* (fig. 11).

Holotype male.—Tecpatan, Chiapas, Mexico, Aug. 30, 1946, at light, A. Dampf. *Paratypes.*—MEXICO: Same data as holotype, 2 ♂. HONDURAS: La Ceiba, May 28, 29, 1949, at light, E. C. Becker, 2 ♂.

This species is extremely similar to *fabae*,



FIGS. 11–17. Parts of male genitalia in *Empoasca*. *A*, *B*, brachone, ventral aspect; *C*, brachone, lateral aspect; *D*, *E*, hook of tenth segment; *F*, styles; *G*, styles and brachones. 11*A*, *B*, two specimens from Illinois; 16*A*, *B*, two specimens from Honduras; 17*A*, *E*, specimen from Honduras, 17*B*, *D*, specimen from Panama.

differing chiefly in the larger apodemes which are much more conspicuous than those of *fabae* because of their heavy mesal sclerotization. In thousands of *fabae* studied, none have been found which exhibit this characteristic.

KEY TO SPECIES OF FABAE COMPLEX
(males only)

1. Base of third tergite with paired crescentic internal apodemes (figs. 1, 2) 2
   Base of third tergite with only linear, inconspicuous apodemes 3
2. Narrowed apex of brachone elongate, about as long as thickened basal portion, and the extreme tip forming an elongate point (fig. 15*A*); lateral areas of tergal apodeme forming only thickened linear areas (fig. 1); sternal apodemes minute (fig. 7) ..... **hastosa**
   Narrowed apex of brachone short, only about half length of basal portion (fig. 17*A*, *B*); lateral areas of tergal apodeme lobate (fig. 2); sternal apodemes large and ovate (fig. 5) ..... **eccla**
3. Bases of sternal apodemes contiguous (figs. 3, 6, 8) 4
   Bases of sternal apodemes well separated (figs. 4, 9, 10) 6
4. Brachone with narrowed apical portion only half length of wide basal portion (fig. 16*A*, *B*); each sternal apodeme slightly constricted at middle, flared at apex (fig. 6, 6*A*) ..... **panisca**
   Brachone with narrowed apical portion nearly as long as free basal portion (figs. 12*A*, 13*A*); sternal apodemes not constricted, hence with virtually parallel sides (figs. 3, 8) 5
5. Sternal apodemes extending little beyond next segmental margin (fig. 8) ..... **mexara**
   Sternal apodemes longer, at least as long as in figure 3 ..... **canda**
6. Sternal apodemes extending only to or not quite to next segmental margin, each apodeme narrower at base and with mesal margin poorly sclerotized and difficult to see, in sharp contrast to the distinct lateral margin (fig. 10) ..... **fabae**
   Sternal apodemes extending markedly beyond next segmental margin, each apodeme moderately thick at base and with the mesal margin as well sclerotized and delineated as the lateral margin (figs. 4, 9) 7
7. Each sternal apodeme subrectangular, the mesal margins nearly straight and parallel with the lateral margins (fig. 4); apex of brachone sinuate, the extreme tip curved laterad (fig. 14*A*, *G*) ..... **kraemeri**
   Each sternal apodeme ovate (fig. 9); apex of brachone curved only mesad (fig. 12*A*) ..... **verdia**

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SUCCINOXIDASE IN THE AMERICAN COCKROACH[1]

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Studies in comparative biochemistry of insect tissues have shown that the absolute levels of certain enzymic functions as well as of several biological products may be correlated with both sex and age of the insect. In some cases, the function increases following the adult molt, whereas in others there may be a decline with age.

Measurements of oxidative metabolism of leg and wing muscles of the woodroach, *Leucophaea maderae* (Fabricius), and of the American cockroach, *Periplaneta americana* (L.), have been reported by several investigators. McShan *et al.*, (1954), in studying three enzyme systems of the pigmented thoracic muscle of *Leucophaea*, found that succinoxidase activity is essentially the same in both sexes but that this system increases with the age of the roach, from 0.5 to 80 days, by about 50%.

Barron and Tahmisian (1948) found the oxygen uptake of skeletal muscle of male American roaches to be approximately twice that of females. Sacktor and Bodenstein (1952) list nearly a two-fold higher level of cytochrome c oxidase in the coxal muscle of male American roaches than in females; and these authors observed that the muscles of the males were red whereas those of females were much lighter. Harvey and Beck, (1953), in a critical study of optimum conditions for succinoxidase measurement of the same roach, between the ages of two to four months, reported a three-fold higher level in coxal muscles of males than of females. Allen and Richards (1954), in comparing the oxidation of succinate by leg muscles of American roaches with that of thoracic muscles of *Tenebrio*

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molitor L. and of *Sarcophaga bullata* Parker, noted a three- to four-fold difference in activity between males and females of the roach, but only a slight difference between groups aged 10 to 20 days and groups aged 95 to 185 days.

Edwards and Perez-Gonzalez (1954) classified all of the muscles of the body in *P. americana* as of a single type, *i. e.*, the "white" type, and found no significance in slight variations of color with sex and age. They concluded that the oxygen consumption, endogenous dehydrogenase, and succinic dehydrogenase activities in leg and flight muscle are similar in the roach and equivalent to the activity of flight muscle in two higher insects. In contrast, Bettini and Boccacci (1954), using triphenyl tetrazolium chloride, reported that succinic dehydrogenase activity does vary between different muscles of the American cockroach. They suggested correlating the visible difference in dye reduction with the difference in muscle pigmentation.

The present study compares the activity of pigmented and non-pigmented muscle and traces in a more quantitative manner the increase in succinoxidase activity with age.

MATERIALS AND METHODS

Freshly-molted individuals of *Periplaneta americana* (L.) were isolated. The instar of the nymphs was estimated according to data of Gier (1947). Because of individual variation in length of instars, it was possible to know only the number of days since the last molt but not the nearness to the next molt.

The muscles were dissected from non-anesthetized insects after longitudinally splitting open the ventral wall of each meso- and metathoracic leg. Dissecting fluid was not used. The tissue was promptly put on ice in a small vessel, the sides and top of which were covered with moist absorbent paper. Subsequent blotting of excess fluid was thus unnecessary.

The muscles were identified (Carbonell, 1947) in adult males of known age and classified as pigmented or non-pigmented regardless of whether they were wing or leg muscles. The pigmentation was a pronounced amber-pink or salmon color as opposed to the translucent white of the non-pigmented bundles. The pink muscles included those of the trochantin; the promotors and

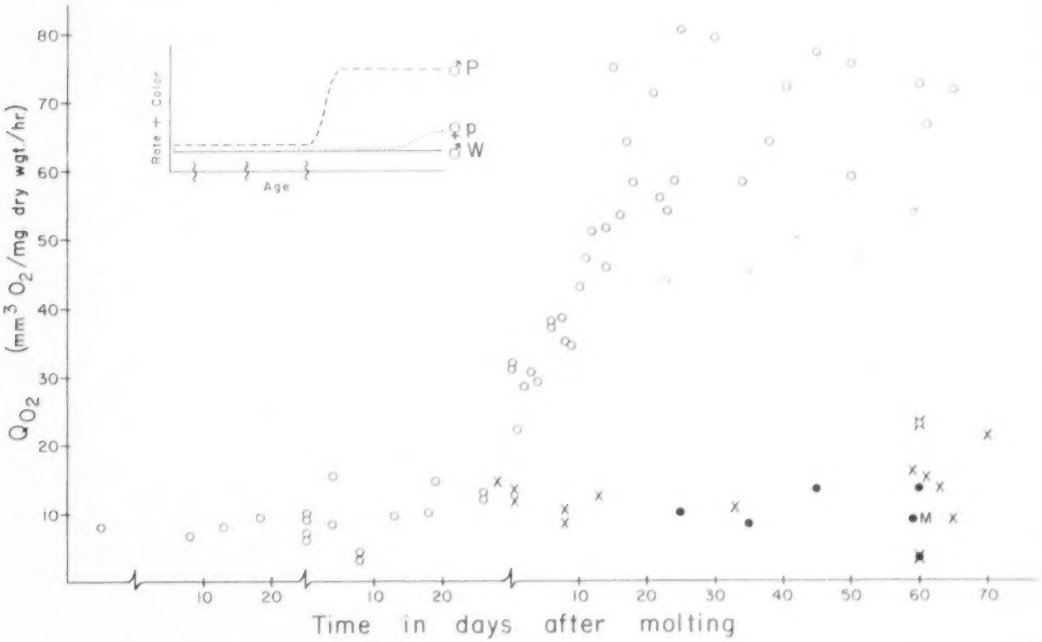

FIG. 1. Large graph shows rate of succinate oxidation as related to age and sex. Abscissa is interrupted at the beginning of the ninth and tenth instars and at the adult molt. The length of each nymphal instar can not be precisely known because of individual variation. Each point represents the average of duplicate determinations on single adults and either single or pooled nymphs. Open circles, pink (or potentially pink) male muscle; solid circles, white male muscle; rayed open circle, malonate-inhibited pink male muscle; rayed solid circle, malonate-inhibited white male muscle; X, female "pink" muscle; M, malonate-inhibited female "pink" muscle.

The small inset is a diagrammatic presentation of the changes in pigmentation and associated enzyme activity as related to sex and age. Dashed line, pink male muscle; dotted line, "pink" female muscle; solid line, white male muscle.

remotors of the coxa; and the a, b, and c branches of the main depressors of the legs. The c branches of the main depressors are called the basalar muscles of the wings, as they originate at the basalar plates, attaching to the apodemal tendons. According to Carbonell, the basalar muscles correspond to the promotor-extensors of the fore- and hind-wings of other insects.

The Carbonell numbers of the pink muscles that were actually used for succinoxidase determinations are as follows: for mesothorax, 116 through 123, 126, 129, 130, 131, and 135 a, b, and c; for metathorax, 154 through 157, 159 through 167, 169, 174, 175, 176, and 177 a, b, and c.

The non-pigmented tissue used from adult males included the anterior and posterior coxal depressors of the legs; the anterior, posterior, and main coxal levators of the legs; and the d and e branches of the main depressors of the legs. (In very old males, there was a slight tinge of pink in the levators and the small branches of the main depressors.)

The Carbonell numbers of the white muscles which were used are as follows: for mesothorax, 135 d and e, 136, 137, 138, 139 a, b, and c, and 140 a, b, c, and d; for metathorax, 177 d and e, 178, 179, 180, 181 a, b, and c, and 182 a, b, c, and d.

All of the muscles in the legs of adult females and in nymphs of both sexes are non-pigmented. The enzyme activity in these groups was measured for only the muscles which are anatomically identical with those that become pink in older adult males. For purposes of identification, these muscles in females and nymphs are called "pink" in this paper even though they are actually colorless.

The wet muscle, usually about 75 milligrams, was weighed to the nearest milligram on a Roller-Smith torsion balance and homogenized while iced in a Potter-Elvehjem vessel as a 5% suspension in de-ionized water. Oxygen uptake in the presence of excess cytochrome c and succinate was measured by the conventional Warburg method at $30 \pm 0.1^{\circ}$ C. The flasks (15 ml. capacity) were charged with 3.0 ml. reactants including 0.05 *M* succinate (recrystallized from the Eastman Organic Chemicals product), 0.05 *M* sodium phosphate buffer pH 7.35, 4×10^{-3} *M* $AlCl_3$, 4×10^{-3} *M* $CaCl_2$, 1×10^{-5} *M* cytochrome c (Sigma Chemical Company), and homogenate equivalent to 7.5 mg. fresh tissue. The rate of oxygen uptake was constant for the first 50 minutes after which it declined. The Qo_2 (expressed as $mm.^3$/mg. dry wt./hour) was calculated on the basis of the 30-minute readings.

RESULTS

The results are summarized in the composite graph of figure 1. The most striking finding was that in males the pink color of the pigmented muscles increased with age while the succinoxidase activity of these same muscles increased also. Therefore there was a positive correlation between pigmentation and enzyme activity. In newly-molted adult males, all of the thoracic muscles were white. Those muscles which were destined to become pigmented gradually did so during the first two weeks following molting, while the muscles in the non-pigmented group remained white. In contrast to the steady rise to a plateau reached at about the 15th day by the pink muscles, the enzyme activity of the white muscles remained at the original low level at least until the 60th day (the age of the oldest male tested).

The Qo_2 of pink male muscle during the first five days following the adult molt averaged 29, ranging between 22 and 32. The Qo_2 of the same muscle between the ages of 15 and 65 days averaged 68, ranging between 44 and 80. This represents an average for aged males of 2.3 times that of young ones, while the figure for the highest individuals is 2.8 times that of the young ones.

The average Qo_2 for white male muscle of all adult ages was 10.4. The value for pink muscle thus ultimately came to 6.5 times that of the white.

All of the thoracic muscles of the adult females were white after molting and for some time thereafter. Although fewer determinations were made for this sex, the range covered the period from the first day to the 70th. The muscles corresponding to the pink muscles of males, from two individuals, one 58 days old and the other 70, had a slight tinge of pink, and the oxygen consumption of these two was higher than that of the others. The average Qo_2 value for the "pink" female muscle was 13.0 ranging from 8.7 to 21.2. Note that the highest figure for aged females is less than the lowest figure for pink muscle of *young* adult males.

Finally, the oxygen consumption of "pink" muscle of male nymphs was somewhat lower, averaging 9.2 and ranging between 3.0 and 19.4. There was no indication of developmental peaks within the instars.

Oxygen uptake of adult muscle without added succinate was negligible. Addition of 10^{-2} *M* malonate inhibited the Qo_2 of male pink muscle by 68%, of male white muscle by 75%, and of female "pink" muscle by 50%.

SUMMARY

Succinoxidase activity in thoracic muscles of the American cockroach is positively correlated with the degree of pigmentation of each muscle. In nymphs of both sexes, all leg and wing muscles are white and have low succinoxidase activity. In the male, soon after the molt to the adult stage, the indirect wing muscles and certain coxal muscles show a great increase in pigmentation and enzyme activity, in two weeks becoming

6.5 times as active as the leg muscles which remain white. In the female, no change is noticeable in either pigmentation or succinoxidase activity until after several weeks of adult life. After about two months, the muscles, which in males become strongly pigmented, become slightly pigmented in the female; and they have slightly higher succinoxidase activity than do those of young females.

Succinoxidase activity, then, is primarily correlated with muscle pigmentation. The pigmentation and correlated enzyme activity change with age in wing and certain leg muscles but not in all leg muscles; and they give rise to a large sex difference by changing sooner and to a much greater extent in the adult male.

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A GROWTH-PROMOTING EFFECT OF CHOLESTEROL IN THE DIET OF LARVAE OF THE HOUSE FLY, MUSCA DOMESTICA L.[1]

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A satisfactory semi-synthetic medium for single-pair culture of the house fly, *Musca domestica* L., has been described (Hammen, 1956). It is a milk-yeast-agar preparation which, on the basis of dry weight of foods, consists of about 38% proteins, 19% lactose, 16.5% glycogen and other polysaccharides, 17% fats, 6.5% minerals, and 3% water. The addition of sucrose to this medium failed to increase survival of adult flies, and in fact inhibited both fecundity and larval growth when presented at aqueous concentrations of 3.5% and above. The experiments reported here represent attempts to assess and improve the medium.

The earlier work showed that when adult flies were placed on an agar medium of yeast alone, they survived only a few days and produced no offspring. On a like medium of milk alone, they survived longer but offspring were few and small. The initial experiment of the present work was intended to determine whether these were failures in adult or in larval nutrition. It was planned also to show whether larval growth is the same on the standard milk-yeast diet and on ground horsemeat, which is the medium on which *Musca* larvae from mass-breeding cultures are reared routinely in this laboratory.

In the comparison with meat, it was apparent that the standard milk-yeast-agar medium does not sustain maximum growth. Since Hobson (1935) first found cholesterol an essential growth factor for larvae of *Lucilia sericata* (Meig.), a like requirement has been noted in all the Diptera, Lepidoptera, and Coleoptera which have been studied in this respect (Trager, 1953). Recently Silverman and Levinson (1954) have shown that the sterol requirement of *Musca* larvae may be satisfied by the sitosterol of wheat bran or by cholesterol derivatives. In further experiments we attempted to improve our medium, therefore, by the addition of pure cholesterol.

MATERIALS AND METHODS

The house flies used in these experiments were derived from a non-resistant stock obtained in 1948 from a commercial laboratory, and inbred on a mass-culture basis for more than 170 generations at 32° C and 35 to 40 percent relative humidity. The standard medium consisted of 100 g dried brewers' yeast, 100 g dried whole milk, 15 g agar, and 3 g methyl *p*-hydroxybenzoate (a mold inhibitor) in one liter of water.

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Experiment 1. The milk medium was identical to the standard, only omitting yeast, and likewise the yeast medium simply lacked milk. All of the media were poured hot into half-pint bottles, a 55 ml. portion in each, and allowed to cool before introducing larvae. The meat medium was ground horsemeat in 55 g portions, placed on a layer of wood shavings in wide-mouth quart jars and in half-pint bottles. Larvae not more than 24 hours old were suspended in 0.8% NaCl, and 0.1 ml samples of them were placed on the various media, then covered with wood shavings to provide a dry place for pupation. The actual numbers of larvae were unknown, but they probably ranged from 170 to 210 in each vessel. The newly emerged adults were weighed while under carbon dioxide anaesthesia in an aluminum foil bucket on a torsion balance.

Experiment 2. A preliminary experiment suggested economy of time and materials in a revised procedure. Instead of measuring them by volume, 20 larvae were counted out and placed in one-fourth-pint bottles on 24 ml of medium representing 4 g of foods. Pure cholesterol was incorporated into the media in dry-weight concentrations of 0, 0.05, 0.10, 0.15, 0.25, and 0.50 per cent. Weights of newly emerged flies were obtained on an analytical balance.

RESULTS

The results of the two principal experiments are summarized in the correspondingly numbered tables. Other results are briefly noted in the discussion.

DISCUSSION

The failure of house flies to produce offspring on yeast alone, in previous work with single-pair matings, is attributable to lack of egg production, since the results of Experiment 1 show that larval growth on yeast equals that on the standard milk-yeast diet. The analogous medium of milk alone, however, proved seriously deficient in one or more factors for larval growth.

Ground horsemeat supported growth conspicuously better than the standard diet. The newly emerged adults from the quart jars were 21.9 percent heavier, and those from the half-pint bottles 12.8 percent. The superiority of the larger containers presumably lay in the relaxation of competition for space.

In preliminary experiments the adult flies which developed from larvae reared on media supplemented with cholesterol were 5 to 6 percent heavier than those from the standard, but there was evidence that the growth-promoting effect was obscured by the effect of crowding. The growth-promoting effect is clearly delineated by the results of Experiment 2. The maximum increase in adult weight, amounting to about 13 percent, is produced at the 0.25 percent level of cholesterol, and sustained at the 0.50 percent level.

The number of larvae reaching adulthood was 991 out of a possible 1300, (Table 2), which is 76.2 per cent survival. Varzandeh, Bruce, and Decker (1954) found 78.7 per cent survival in a comparable strain of house flies. The most viable strain investigated by Babers, Pratt, and Williams (1953) had 78.9 percent survival from larva to adult.

According to Nataf, Mickelsen, Keys, and Petersen (1948), the cholesterol content of cows' milk has a mean value of 11.3 mg per 100 ml. Dividing by the total solids in milk, 12.8 g per 100 ml as reported by Macy, Kelley, and Sloan (1953), gives 0.088 percent cholesterol in dried milk. This agrees precisely with the figure given by Lange (1950) of 88 mg per 100 g milk powder. Thus the addition of 0.04 to the percentages recorded gives the total cholesterol content of the milk-containing media in the present study.

The sterol produced by yeast, according to Breivik, Owades, and Light (1954), is predominantly (up to 80 percent) ergosterol. Light (1955) has stated that the type of brewers' yeast used in these experiments has a sterol content of 1.5 to 2.5 percent, of which about 60 percent is ergosterol, 30 percent zymosterol, and the remaining 10 percent composed of small quantities of ascosterol, fecosterol, 5-dihydroergosterol, and 24(28)-dehydroergosterol.

These facts permit the conclusion that a diet containing approximately 1.0 percent ergosterol and related yeast sterols sustains complete development of house fly larvae, but is inadequate for maximum growth, and is markedly improved

TABLE 1

GROWTH OF LARVAE TO ADULTS ON VARIOUS MEDIA (5 BOTTLES OF EACH).

MEDIUM	ADULT FLIES EMERGING	FLIES PER BOTTLE	TOTAL WEIGHT (g)	MEAN WEIGHT PER FLY (mg) ±S.E. (PER BOTTLE)	PERCENT DIFFERENCE IN MEAN WEIGHT
standard	714	142 8±3 6	9 493	13 31±0 18	0
milk	23	4 7±1 8	0 194	7 83±0 85	−41 2
yeast	644	128 8±2 2	8 498	13 20±0 10	0
meat (quarts)	791	158 2±4 3	12 823	16 23±0 20	21 9
meat (half-pints)	762	152 4±8 4	11 442	15 02±0 12	12 8

by the presence of cholesterol in a total concentration of 0.29 percent of the dry weight of foods. This agrees with the observation of Hobson that ergosterol is less active than cholesterol in promoting larval growth of *Lucilia*, and with that of Brust and Fraenkel (1955) that both growth rate and final weights of larvae of *Phormia regina* (Meig.) are less on diets containing ergosterol than on cholesterol diets. According to Hobson, page 2024, "Since blow-fly larvae normally breed only in animal tissues, it is not surprising to find that they utilize cholesterol more readily than sterols from other sources".

The minimum concentration of cholesterol which supported satisfactory growth of *Lucilia* larvae for 2½ days at 35° C was given by Hobson as 0.30 percent of the dry weight of the diet. Brust and Fraenkel found that 0.25 percent cholesterol was just as effective as higher concentrations in promoting growth of *Phormia* larvae over a period of 7 days at 29° C. For best growth of *Musca* larvae to pupation in 5 days at 32° C, this report establishes the minimum concentration of cholesterol as 0.29 percent. This agreement among species on a narrow range of concentrations at which all exhibit their best growth probably has an adaptive significance. The cholesterol content of fresh mammalian muscle, according to West and Todd (1951), is 0.07 percent of the total weight, or, using their estimate of 25 percent solids, 0.28 percent of the dry weight. Since *Lucilia*, *Phormia*, and *Musca* commonly breed in carrion, their growth response to this level of cholesterol is probably a dietary adaptation produced by the selective influence of a favored environment.

SUMMARY AND CONCLUSIONS

1. Larvae of *Musca domestica* grow satisfactorily on a non-sterile agar-base medium of brewers' yeast alone, indicating that this is a complete or nearly complete food; a similar medium of dried milk alone is clearly inadequate.

2. The larvae develop into larger adults when reared on ground horsemeat than when reared on a standard milk-yeast-agar medium.

3. They also develop into larger adults when the standard diet is supplemented with pure cholesterol; the minimum concentration of cholesterol for best growth, when superimposed on approximately 1.0 percent yeast sterols already present in the diet, is 0.29 percent of the dry weight of foods.

4. The growth responses of larvae of *Musca domestica* and two species of blow-flies to a cholesterol concentration approximating that in fresh mammalian muscle may be regarded as a dietary adaptation to carrion breeding.

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TABLE II

GROWTH OF LARVAE TO ADULTS ON MILK-YEAST-AGAR MEDIUM SUPPLEMENTED WITH CHOLESTEROL.

PERCENT CHOLESTEROL	LARVAE	ADULT FLIES	TOTAL WEIGHT (g)	MEAN WEIGHT PER FLY (mg) ±S.E. (PER BOTTLE)	PERCENT INCREASE IN MEAN WEIGHT
0	240	191	2.5485	13.33±0.22	0
0.05	300	206	2.7904	13.55±0.23	1.6
0.10	360	264	3.7073	14.03±0.16	5.2
0.15	200	169	2.4189	14.31±0.16	7.4
0.25	100	87	1.3170	15.16±0.19	13.7
0.50	100	74	1.1162	15.06±0.33	13.0

EFFECTS OF ADDED SUBSTRATES AND INHIBITORS ON HOMOGENATE RESPIRATION DURING THE METAMORPHOSIS OF THE MEALWORM, TENEBRIO MOLITOR LINNAEUS[1]

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Since the work of Potter and Elvehjem (1936), tissue homogenates have been used extensively for the study of the enzymatic activity of vertebrate tissues. The homogenate technique was applied to a study of insects by Bodine and Lu (1950). They homogenized embryos of the grasshopper, *Melanoplus differentialis* (Thos.), and compared the rate of O_2 consumption of whole embryos with that of their homogenates during diapause and non-diapause. Bodine, Lu, and West (1952) extended these experiments to include the effects of adding substrates and inhibitors to homogenates of grasshopper embryos. These experiments showed marked differences in the enzymatic activity of diapause and non-diapause stages.

The use of the homogenate technique should give valuable information concerning the relative importance of various enzyme systems at different periods of metamorphosis. Krogh (1914) first described a U-shaped respiratory curve as being characteristic of this process. Fink (1925), and Needham (1942), correlated this respiratory curve with the degree of tissue organization. They suggested that the amount of disorganization is greatest at the low point of the respiratory curve. On the other hand, a group of investigators (Wolsky, 1938 and 1941; Agrell, 1949; Williams, 1950; Sacktor, 1951; and Ludwig, 1953) found U-shaped activity curves during the pupal stage for cytochrome oxidase and various dehydrogenases. They correlated the activities of these enzymes with the rate of O_2 consumption. The activity curves of cytochrome oxidase and succinic dehydrogenase are not U-shaped during the metamorphosis of the mealworm, *Tenebrio molitor* (Ludwig and Barsa, 1956a), although the respiratory curve is U-shaped in this species. Hence, a study of the O_2 consumption of mealworm homogenates with the addition of various substrates and inhibitors was made to determine the relative importance of these two enzyme systems. Two homogenate concentrations (1 and 5 percent) were used because Ludwig and Barsa (1956b) found that endogenous metabolism varied with homogenate concentration.

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MATERIAL AND METHODS

Prepupae, obtained from a mixed culture maintained at room temperature, were placed in beakers at 30° C. and examined daily. Newly molted pupae were collected at approximately the same time each day, and in this way, timed pupae (within 24 hours) were obtained. The pupae were kept in beakers at 30° C. until they reached the desired stage of metamorphosis. Determinations of O_2 consumption were made on prepupae, pupae for each day of the pupal stage, and on newly emerged adults. The insects were homogenized for 1 minute in a motor-driven glass homogenizer in 0.03 molar phosphate buffer adjusted to a pH of 7.4. Readings on the rate of O_2 consumption were made over a 2-hour period in Warburg constant volume manometers, with 15 ml. flasks, at 30° C. according to the method outlined by Umbreit, Burris, and Stauffer (1945). Throughout this period, the manometers were rocked horizontally 100 times per minute.

The following determinations were made: (1) endogenous metabolism (unfortified homogenate); (2) metabolism of 1 percent and of 5 percent homogenates with the addition of the substrates, cytochrome *c*, sodium succinate, or both; and (3) metabolism of 1 percent and of 5 percent homogenates, with and without the added substrates, and with KCN, sodium malonate, or DDT. The final concentrations of the substrates in the manometer flasks were:

(a) 0.6×10^{-4} M cytochrome *c*,
(b) M/8 sodium succinate,
(c) 0.6×10^{-4} M cytochrome *c* plus M/8 sodium succinate.

The inhibitors were used in the following final concentrations:

(a) 10^{-4} M KCN,
(b) M/8 sodium malonate,
(c) 10^{-3} M DDT in 1.25 percent ethyl alcohol.

The DDT was prepared by dissolving the crystals in 5 ml. of absolute ethyl alcohol and adding this solution to 95 ml. of phosphate buffer. Cyanide inhibition was determined by the method of Robbie (1946). All solutions were made in 0.03 molar phosphate buffer adjusted to a pH of 7.4. In each experiment, the thermobarometer contained all solutions except the tissue homogenate.

OBSERVATIONS

The rate of O_2 consumption of 5 percent

homogenates is shown in Graph A, Figure 1. Each point is an average of at least 25 determinations. Endogenous metabolism occurred at a relatively constant level throughout metamorphosis, but was higher in newly emerged adults.

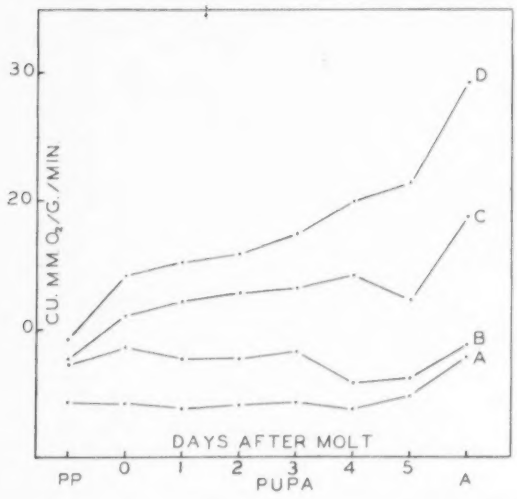

FIG. 1. O_2 consumption of 5 percent homogenates during metamorphosis. Graph A, endogenous metabolism; B, homogenate with cytochrome *c*; C, homogenate with sodium succinate; D, homogenate with cytochrome *c* and sodium succinate. PP, prepupa; P, pupa; A, newly emerged adult.

However, it was very variable. An attempt was made to determine factors responsible for this variation. The factors studied were parental age and the duration of the larval stage. Homogenates of pupae obtained from eggs laid by recently emerged beetles had a respiratory rate which was not significantly different from that of pupae obtained from eggs laid by the same beetles more than 6 weeks later. Homogenates of pupae which had required less than 5 months for larval development used oxygen at a somewhat, but not significantly, higher rate than those of pupae which had required more than 7 months as larvae. An interesting observation was that those homogenates with high rates of endogenous metabolism usually blackened during the course of the experiment. The O_2 consumption of homogenates which became black was approximately twice that of others which remained colorless.

The effects of adding substrates on the respiratory rate of 5 percent homogenates are shown in Graphs B, C, and D, of Figure 1. Each point of these graphs is an average of at least 15 determinations. The addition of cytochrome *c* (Graph B) increased the respiratory rate throughout the period of metamorphosis. However, the amount of this increase was greater in the prepupal and the early part of the pupal stage than it was during the last 2 days of the pupal period or in the newly emerged adult. The addition of sodium succinate (Graph C) increased the rate of O_2 consumption throughout the period of metamorphosis. In this case, the increase was not as great in the prepupa as it was during the pupal stage. When both cytochrome *c* and succinate were added to homogenates (Graph D), the respiratory rate was increased to a greater degree than with either substrate alone. With both substrates, the rate of O_2 uptake showed a progressive increase throughout metamorphosis.

The addition of any of the inhibitors, KCN, sodium malonate, or DDT, did not change the respiratory rate of unfortified homogenates at any stage of metamorphosis. The effects of adding these inhibitors to 5 percent homogenates with added substrates are shown in Figure 2. Graph C shows the respiratory rate of the homogenate with added cytochrome *c* and succinate. Graph A represents the O_2 consumption of the homogenate with these added substrates and with KCN; and Graph B, with sodium malonate. Each point of Graphs A, B, and C, is an average

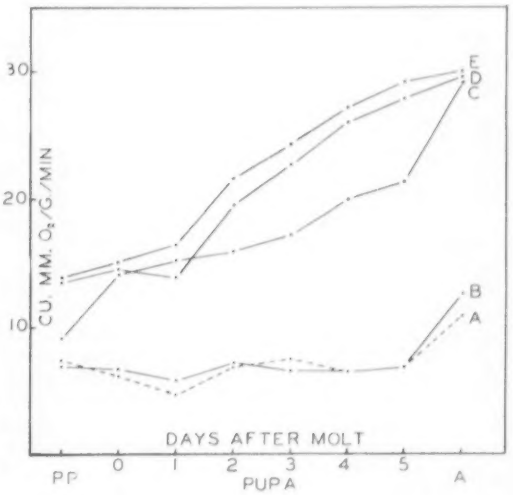

FIG. 2. O_2 consumption of 5 percent homogenates with added substrates and inhibitors. Graph A, homogenate with cytochrome *c*, sodium succinate, and KCN; B, homogenate with cytochrome *c*, sodium succinate, and sodium malonate; C, homogenate with cytochrome *c* and sodium succinate; D, homogenate with cytochrome *c*, sodium succinate, 1.25 percent alcohol, and DDT; E, homogenate with cytochrome *c*, sodium succinate, and 1.25 percent alcohol. PP, prepupa; P, pupa; A, adult.

of at least 15 readings. Each of the inhibitors in the concentrations used in these experiments (10^{-4} M KCN, M/8 sodium malonate) eliminated nearly all of the respiration induced by the addition of substrates. Since the addition of cytochrome *c* and succinate to 5 percent homo-

genates resulted in a progressive increase in respiratory rate throughout metamorphosis, the inhibitors KCN and malonate showed progressively greater amounts of inhibition. The effects of adding DDT to 5 percent homogenates with added substrates are shown by comparison of

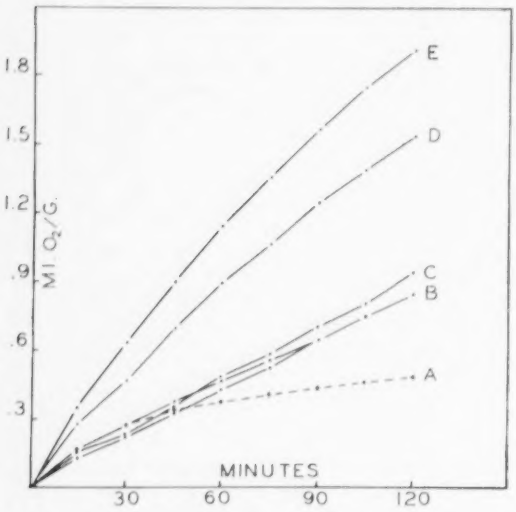

FIG. 3. O_2 consumption of 5 percent homogenates of 2-day pupae. Graph A, endogenous metabolism; B, homogenate with cytochrome *c*, sodium succinate, and either sodium malonate or KCN; C, homogenate with cytochrome *c*; D, homogenate with sodium succinate; E, homogenate with cytochrome *c* and sodium succinate.

Graphs D and E. Each point in these graphs is an average of at least 10 determinations. The effect of adding alcohol to the homogenate is shown in Graph E. These values serve as controls for the study of DDT inhibition (Graph D). The addition of alcohol in 1.25 percent increased the respiration of homogenates (with substrates) of 2- to 5-day pupae. DDT in 10^{-3} molar concentration produced a slight inhibition of approximately 6 percent, which although not significant, was present throughout metamorphosis.

The O_2 consumption of 5 percent homogenates of 2-day pupae over a 2-hour period is shown in Figure 3. Similar graphs were constructed for each day of pupal development with essentially the same relationships. The respiratory rates of unfortified homogenates decreased progressively with time, while those of homogenates with cytochrome *c*, succinate, or with both cytochrome *c* and succinate were higher and were maintained nearly constant throughout the observation period. The inhibitors KCN or malonate when added to homogenates containing both substrates, resulted in a respiratory rate which was also maintained constant but at a much lower rate. With inhibitors, the rate was either lower than (results not plotted), or the same as, that of the unfortified homogenate early in the observation period; but at the end of 2 hours, it was greater. This observation suggests that the decreasing rate of endogenous metabolism is associated with a decrease in available substrate.

The rates of O_2 consumption of 1 percent homogenates, with added substrates and inhibitors are shown in Figure 4. All of the points represent an average of at least 10, except in Graphs D and G, where a minimum of 22 readings were made. The metabolic rate of unfortified 1 percent homogenates (Graph B) was relatively constant throughout metamorphosis although at a lower rate than that of 5 percent. However, with the addition of cytochrome *c* and succinate, it was greater in 1 percent. The addition of these substrates thus resulted in a greatly increased rate of O_2 consumption, the amount becoming increasingly greater as metamorphosis progressed (Graph

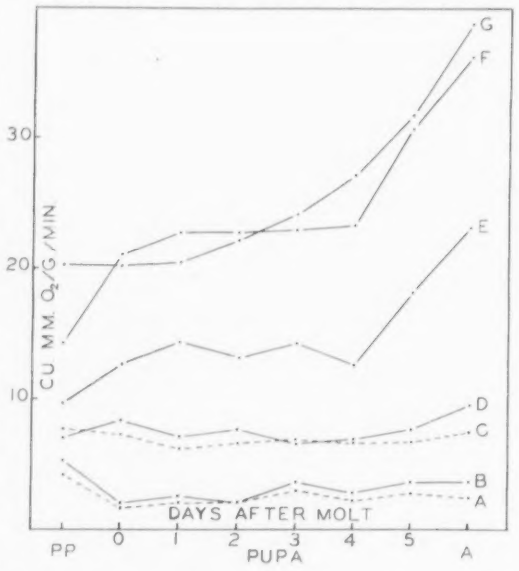

FIG. 4. O_2 consumption of 1 percent homogenates during metamorphosis. Graph A, homogenate with KCN; B, endogenous metabolism; C, homogenate with cytochrome *c*, sodium succinate, and sodium malonate; D, homogenate with cytochrome *c*, sodium succinate, and KCN; E, homogenate with cytochrome *c*, sodium succinate, 1.25 percent alcohol, and DDT; F, homogenate with cytochrome *c*, sodium succinate, and 1.25 percent alcohol; G, homogenate with cytochrome *c* and sodium succinate. PP, prepupa; P, pupa; A, adult.

G). None of the inhibitors were effective when added to unfortified homogenate. Graph A shows the respiratory rate of unfortified homogenate with 10^{-4} M KCN. The values plotted in this graph are not significantly different from

those in Graph B. The addition of sodium malonate or of KCN to homogenates with cytochrome *c* and succinate resulted in an inhibition of approximately 90 percent of the added respiration (Graphs C and D). The addition of 1.25 percent alcohol to homogenates with both substrates did not significantly change the rate of O_2 consumption. However, when 10^{-3} M DDT was added to homogenates with the added substrates, the rate was reduced by approximately 39 percent throughout metamorphosis (Graph E).

DISCUSSION

Cotty (1955) studied the effect of adding substrates to homogenates of the house fly, *Musca domestica* L., during metamorphosis. He found no effect on adding cytochrome *c*. In the present experiments, its addition produced an increased O_2 consumption. Cotty's observations were made over a 1-hour, instead of a 2-hour period. Figure 3 shows that with homogenates of 2-day mealworm pupae, adding cytochrome *c* had little effect until the second hour. Results obtained for other days of pupal development also show the same relationship. They are therefore in essential agreement with those of Cotty. The increased respiration induced by the addition of cytochrome *c* during the early part of the pupal stage (Graph B in Figure 1) indicates that there may be a deficiency of this compound early in metamorphosis. The fact that its addition had only a slight effect in later stages demonstrates that its concentration increases towards the end of this process. These results are comparable to those of Williams (1950) who found that in the silkworm, *Platysamia cecropia* (L.), there is a deficiency in the cytochrome system, both cytochrome *c* and cytochrome oxidase, at the low point in respiratory activity. Since the addition of cytochrome *c* did not produce a significant increase in the O_2 uptake of homogenates of 4- or 5-day pupae, or of adults, the limiting factor appears to be substrate concentration. Thus it appears that the available succinate in the homogenate decreases during metamorphosis. Handler and Klein (1942) found that DPN is rapidly broken down by tissue homogenates. The destruction of this coenzyme would block the formation of succinate from other substrates. Hence, conditions existing in homogenates may be very different from those in intact insects.

The addition of succinate alone, or of succinate with cytochrome *c* to homogenates resulted in a rate of O_2 consumption which was greater than that obtained by Ludwig and Barsa (1956b) for intact pupae and adults of *Tenebrio molitor*. In their experiments, a U-shaped curve was obtained with values of 9.8 μ liters/gram/minute in newly molted pupae; 4.7, in 2-day pupae; and 18.5, in newly emerged adults. The corresponding readings for 5 percent homogenates with the added substrates were 14.1, 16.0, and 29.3, respectively. Homogenization in hypotonic media may result in a rupture of cell structures, such as the mitochondria, so that the respiratory enzymes are freed into the solution and thus are brought into contact with the available substrate. These results are not in accord with those of Cotty (1955) who found an increase in the rate of O_2 consumption of house fly homogenates with the addition of cytochrome *c* and succinate, although the rate was considerably lower than that of intact pupae. It is probable that pupae of the mealworm have a greater excess of the enzymes of the succinoxidase system than do those of the house fly.

Cotty (1955) found that the addition of cytochrome *c* and succinate to homogenates of house fly pupae during metamorphosis resulted in a restoration of the U-shaped respiratory curve which had been eliminated as a result of homogenization. In the present experiments, the addition of these substrates did not restore the U-shaped curve. In the house fly, Sacktor (1951) found that the curve of cytochrome oxidase activity was also U-shaped during metamorphosis. Ludwig and Barsa (1956a) reported that the activities of cytochrome oxidase and succinic dehydrogenase were low during the early part of the pupal stage in the mealworm and increased during the latter part of this stage. Similar results were obtained by Ito (1955) for the activities of cytochrome oxidase and of the succinoxidase system of the silkworm, *Bombyx mori* L. Thus in both the mealworm and in the silkworm, the U-shaped respiratory curves are not determined by the activities of cytochrome oxidase nor of succinic dehydrogenase.

Sodium malonate is a competitive inhibitor of succinic dehydrogenase and KCN inhibits cytochrome oxidase. The observation that these inhibitors did not effect the O_2 consumption of unfortified homogenates of mealworm pupae may indicate that their endogenous metabolism does not involve the succinoxidase system. The addition of either of these inhibitors to homogenates containing cytochrome *c* and succinate produced the same amount of inhibition throughout metamorphosis although different parts of the succinoxidase system are affected by each. DDT inhibits both enzymes as indicated by the experiments of Anderson, March, and Metcalf (1954), who obtained a DDT inhibition of cytochrome oxidase, succinic dehydrogenase, and of the succinoxidase system of the house fly; and of Ludwig, Barsa, and Cali (1955), who showed a DDT inhibition of cytochrome oxidase which varied with homogenate concentration. In the present experiments, the extent of inhibition also varied with homogenate concentration, only a slight amount occurring with 5 percent, and approximately 39 percent with 1 percent homogenates. Ludwig, Barsa, and Cali suggested that the higher homogenate concentrations may have an excess of enzyme so that a large proportion of

enzyme activity could be eliminated without decreasing O_2 consumption.

SUMMARY

1. The effects of adding substrates and inhibitors on the O_2 consumption of mealworm homogenates were determined throughout metamorphosis.

2. Endogenous metabolism of 5 percent homogenates occurred at a constant low rate throughout the pupal stage. Readings were variable. Neither parental age nor the duration of the larval period were important in modifying endogenous metabolism of pupae. Homogenates with a high rate of O_2 consumption blackened during the observation period.

3. The addition of cytochrome *c* produced a considerable increase in the O_2 consumption of prepupae and pupae during the first part of the pupal period, but only a slight increase in late pupae and in newly emerged adults. This effect was not evident until the second hour after its addition. The results indicate a deficiency of cytochrome *c* early in metamorphosis when the respiratory rate of intact pupae is low.

4. The addition of sodium succinate alone to 5 percent homogenates produced an increase in O_2 consumption which became progressively greater during the pupal stage. The addition of both cytochrome *c* and succinate resulted in a still greater progressive increase in respiratory rate. In each case, the rate was greater than that of the intact pupa. These substrates did not restore the U-shaped respiratory curve characteristic of intact insects. Hence, the enzymes of the succinoxidase system are not limiting factors determining respiratory rate during the early part of the pupal stage of this species.

5. The inhibitors, KCN, sodium malonate, and DDT, had no effect on endogenous metabolism which, therefore, appears not to be mediated through the succinoxidase system. In the concentrations used in these experiments, KCN and malonate each inhibited nearly all of the O_2 uptake induced by the addition of cytochrome *c* and succinate. The addition of 10^{-3} M DDT resulted in a slight (approximately 6 per cent) inhibition of the metabolism of 5 percent homogenates with the added substrates, which was constant throughout metamorphosis.

6. The endogenous metabolism of 1 percent homogenates was also constant throughout metamorphosis but at a lower rate than that of 5 percent homogenates. However, the addition of the substrates, cytochrome *c* and succinate, produced a greater increase in the O_2 uptake of 1 percent homogenates. KCN and sodium malonate each inhibited approximately 90 percent of this added respiration. DDT in 10^{-3} molar concentration inhibited approximately 39 percent of the O_2 consumption of 1 percent homogenates with the added substrates.

7. The addition of 1.25 percent alcohol increased the respiration of 5 percent homogenates throughout the pupal stage, except for newly molted pupae, but had no effect on that of 1 percent homogenates, each with the added substrates, cytochrome *c* and sodium succinate.

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THE EMBRYONIC DEVELOPMENT OF THE CORN EARWORM, HELIOTHIS ZEA (BODDIE)[1] (LEPIDOPTERA, PHALAENIDAE)

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INTRODUCTION

Although considerable work has been done on the embryology of various Lepidoptera, a complete account of the development of a phalaenid is not available in the literature. In addition, this information should prove to be of value from an economic standpoint since the earworm is an important agricultural pest in the United States. For these reasons the present work on the corn earworm, *Heliothis zea* (Boddie) was carried out.

The use of ovicides in controlling infestations of earworm has also shown promise, particularly on corn (Raffensperger and Rutschky 1953), and a study of embryonic development is desirable in understanding the processes of ovicidal action.

Tietz (1952) listed 101 species of plants that may serve as food, the most important of which are corn, tomatoes, cotton, beans and tobacco. On corn, the females prefer the silks as a site for deposition of the eggs. These are a pale yellow green in color at oviposition, becoming darker as development proceeds. In the field the eggs hatch in from 3 to 4 days. In the laboratory, under a constant temperature of 25° C. hatching occurred from 80 to 82 hours.

SURVEY OF PERTINENT LITERATURE ON THE EMBRYOLOGY OF LEPIDOPTERA

The embryology of the Lepidoptera as a group has been widely investigated ever since Malpighi (1669) studied the development of the silkworm, *Bombyx mori* (L). Johannsen and Butt (1941) list 149 papers dealing with lepidopteran embryology up to 1940, a large number of which (51) are devoted to the silkworm. Christensen (1942) has presented a good historical review of the important papers in the field.

The only published descriptions of phalaenid embryology are those of Hirschler (1905, 1907), Gross and Howland (1940) and of Rempel (1951). Hirschler was primarily concerned with blastokinensis and inner layer and mid-gut formation. In his first paper he dealt solely with *Catocala nupta* L. In the second he studied several species: *Catocala nupta* L., *C. fraxini* L., *Ennomos quercinaria* Hufn. and *Cidaria ocellata* L. The paper by Rempel on *Mamestra configurata* Walk. is much broader in scope but carries development only as far as blastokinensis while that by Gross and Howland on *Prodenia eridania* (Cram.) deals only with the early stages of development as far as gastrulation. Doring (1936–39) was concerned with the sculpturing of the chorion and micropyle region of the eggs of a number of species of Phalaenidae.

In addition to the above papers, dealing directly with phalaenids, those of Johannsen on *Diacrisia virginica* (F.) (1929) and Eastham on *Pieris rapae* (L.) (1927, 1930) should be mentioned. These constitute very thorough treatments of the subject and the development of *Heliothis* is quite similar to these forms in many particulars.

The embryology of the earworm itself is unreported except for a brief mention in Quaintance and Brues (1905). The statements made there were clearly based on observation of living eggs and give the time of completion of the blastoderm, appearance of the germ band and appendages, and deposition of pigment in the integument.

METHODS AND MATERIALS

To provide eggs for the work, a culture of earworms was maintained during the late summer and part of the winter in both 1952 and 1953. As the adults emerged they were paired in oviposition cages. For oviposition, strips of cheesecloth 1½ by 4 inches were hung along the walls of the cages; the rectangular wire frames developed by Raffensperger (1952) were also used.

During the period of oviposition the material on which the eggs were being deposited was examined every 20 minutes. Strips of material holding eggs were removed and placed in a constant temperature cabinet maintained at 25° C. The eggs were removed from the strips for fixation as needed, fixed in hot formalin-acetic acid-alcohol mixture (60° C.), and stored in 70% alcohol.

After the eggs had been stored in 70% alcohol the chorions were removed and the embryos stained in borax carmine. The embryos could then be studied "in toto" or imbedded in paraffin and sectioned. In the preparation of sections a fairly hard grade of Fisher's Tissuemat (56–58° C.) was used for infiltration and imbedding. All sections were cut at 4μ and stained with Heidenhain's iron hematoxylin. Some of the slides were counterstained with eosin. Damar was used as a mounting medium.

Some of the chorions which had been removed

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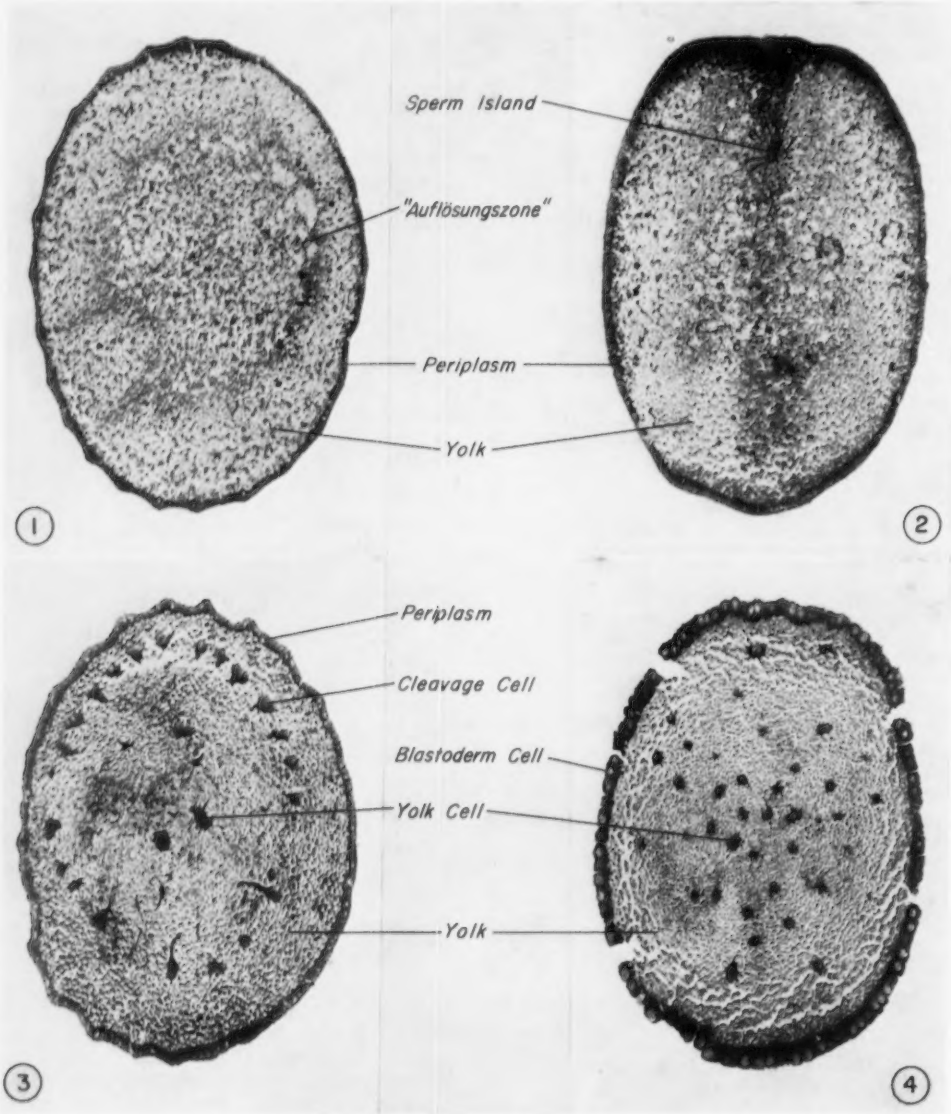
from eggs were stained in an aqueous solution of acid fuchsin. These preparations were used for examining the micropyle. In a few cases the chorions were not removed from the eggs. These eggs were doubly imbedded in celloidin-paraffin and sectioned at 5 μ. The sections were treated with Kennedy's (1932) modification of Mallory's connective tissue stain and used for examining the finer structure of the chorion.

THE EGG

The egg of the earworm is nearly globular in form with flattened anterior and posterior ends. It has an average equatorial diameter of 0.6 mm.

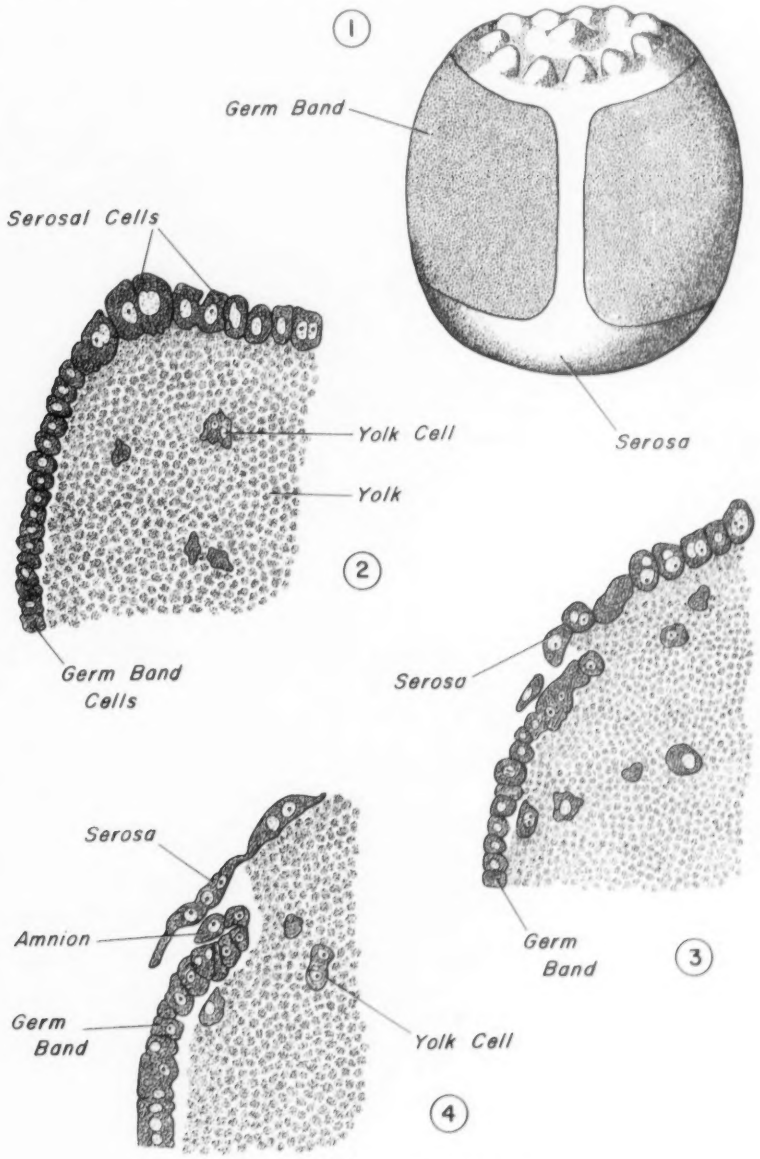


EXPLANATION OF PLATE I

1. Cross section through posterior region of 30 minute egg. Note division of yolk into three regions. × 168, photomicrograph. 2. Longitudinal section through 30 minute egg. × 168, photomicrograph. 3. Cross section through 4 hour egg, showing cleavage cells approaching periplasm. × 168, photomicrograph. 4. Longitudinal section through 5 hour egg, showing blastoderm forming with cell membranes not yet complete on surface facing yolk. × 168, photomicrograph.

The characteristic sculpturing of the chorion is described in detail by Quaintance and Brues. Directly in the center of the circular area at the anterior pole, on a low, raised projection, is the single micropyle. The micropylar opening can be seen very clearly in chorions which have been removed from the eggs and stained with acid fuchsin. It is circular in shape and measures about 7μ in diameter.

Cross sections of the chorion, treated with



EXPLANATION OF PLATE II

1. Surface view of 6½ hour egg from anterolateral aspect with chorion removed, showing blastoderm differentiated into germ band and serosa. × 86. 2. Longitudinal section through anterior region of 6 hour egg, showing difference between germ band and serosa cells. × 300. 3. Longitudinal section through anterior region of 6½ hour egg, showing serosa expanding over germ band. × 269. 4. Longitudinal section through anterior region of 8 hour egg, showing start of amnion formation. × 244.

Mallory's connective tissue stain, show a distinctly laminated structure. The entire chorion can be divided into two general layers; an inner endochorion and an outer exochorion. The endochorion is a fairly uniform, light orange staining layer measuring 1.5 μ in thickness. Along its outer margin is a very thin deeper orange layer. The exochorion also consists of two layers: an inner blue layer, next to the endochorion, and an outer, very thin, dark blue layer. The inner layer of the exochorion varies considerably in thickness, being thickest in the regions between the longitudinal ridges.

The periplasm averages 6.8 μ thick except at the anterior pole of the egg where it is somewhat thicker (Pl. I, fig. 2). As described by various authors the periplasm is divided into two distinct regions; an inner, more dense, non-vacuolated cytoplasm and an outer, granular, strongly vacuated cytoplasm.

Structurally, the yolk presents a rather complex picture. It consists of three well defined regions (Pl. I, figs. 2, 3), the central core, the "Auflösungszone", and an outer zone. The central core consists of a mass of light staining, irregularly shaped globules interspersed with numerous vacuoles; the vacuoles presumably were left by the removal of soluble fats and oils during the preparation of the sections. Interspersed through this region of the yolk is a meshwork of dark staining cytoplasmic strands which sometimes completely encircle the yolk spherules.

Encircling this central core is a band of yolk in which the yolk spherules have merged to form a denser, granular mass. This region is less highly vacuolated and shows fewer cytoplasmic strands than the central core. This region was termed the "Auflösungszone", or zone of solutions, by Christensen (1942), who believes that the shape of this region may be characteristic for many species. In *Heliothis* it is not present beneath the anterior pole and thus in longitudinal section is "U" shaped.

The outer region lies between the "Auflösungszone" and the periplasm. The yolk in this region is similar in character to that of the central core except that it becomes somewhat denser in its outermost layers, adjacent to the periplasm.

The yolk in 15-minute old eggs has a varying number of very dense, blue black staining spherules. These vary from 2 to 5 μ in diameter and most of them are surrounded by a distinct layer of lighter staining cytoplasm. In some cases the layer is quite thick. They are concentrated in the posterior portion of the egg and are restricted almost entirely to the zone of solution described above. The spherules vary somewhat in their staining reaction, some of them staining even more lightly than the surrounding cytoplasm. Similar structures were also described by Gross and Howland in the eggs of *Prodenia eridania* (Cram.) We believe that they may be the remains of a synthethic center, carried over from the developing ova, similar to that described by Payne (1932) in the ova of six orders of insects. In *Heliothis*, the spherules remain present in the egg during the first 1½ hours of development and then disappear.

The yolk, after the passage of the cleavage nuclei through it, becomes more homogenous in nature and neither the three regions nor the cytoplasmic reticulum can be distinguished. In spite of the fact that the yolk no longer appears morphologically differentiated after passage of the cleavage nuclei, there is some indication that it remains physiologically differentiated. This is because the yolk nuclei, a few hours after formation of the blastoderm, become localized in definite regions of the yolk. Most of them become localized in what was formerly the middle, more granular, "Auflösungszone." A smaller number are located in the center of the egg and around the periphery, close to the blastoderm. At the same time, many dark staining spherules again appear in the yolk and also are almost entirely restricted to these same regions. The restriction of the spherules to areas close to the yolk cells indicate that these cells have assumed a secretory activity and are altering the yolk chemically, preparing it for assimilation by the developing germ band. The distribution of the spherules rapidly becomes more diffuse, spreading through the entire center yolk by 9 hours. By this time also, the yolk begins to break up into yolk balls, a process which begins at the periphery and works toward the center, being completed about three hours later. With the formation of the yolk balls, differentiation of the yolk into definite regions is again lost. Dark spherules are still present, however, concentrated around the yolk nuclei at the center of each yolk ball.

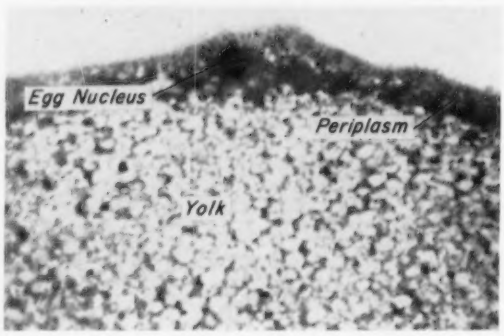
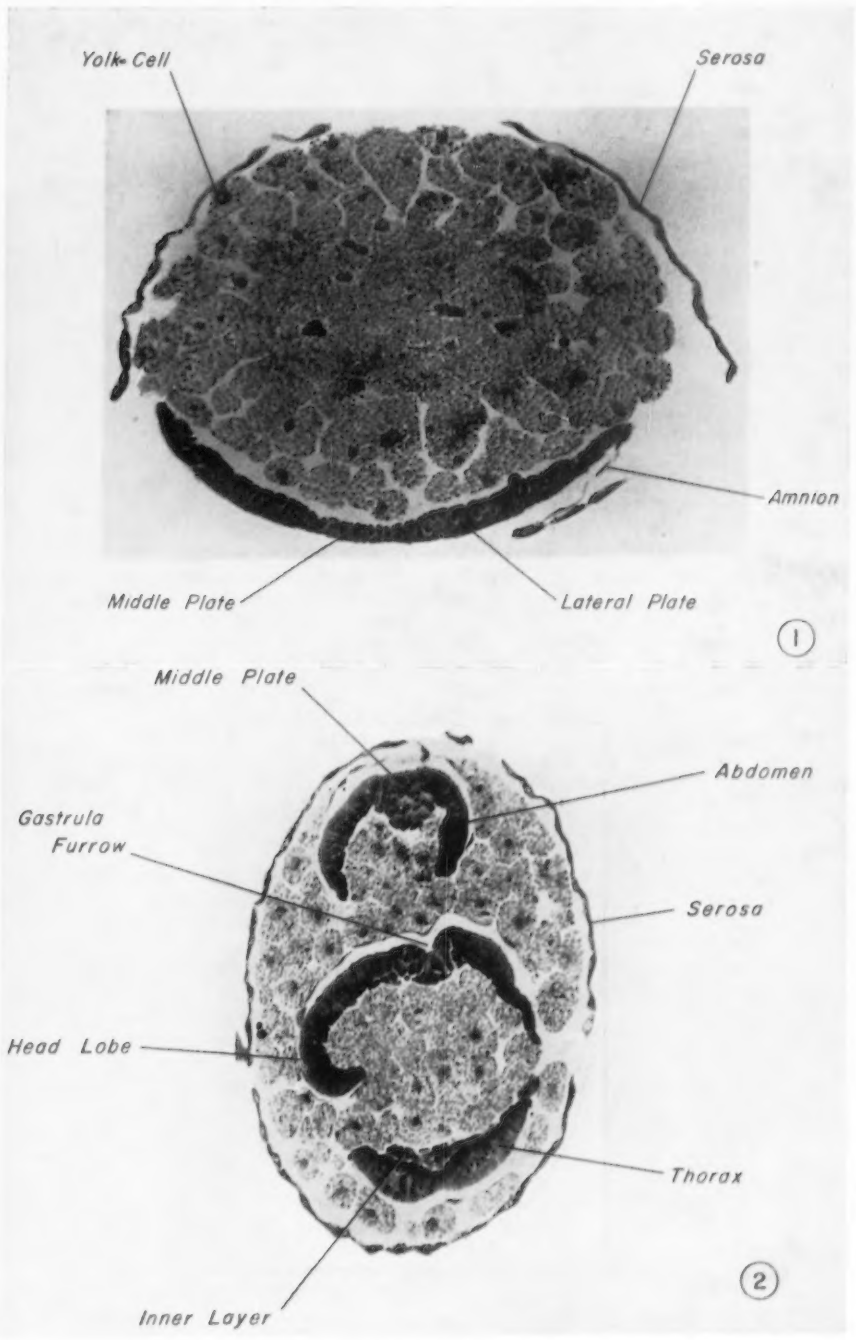


FIG. 1. Longitudinal section through portion of periplasm in anterior region of 15 minute egg; egg nucleus in telophase of first reduction division. × 455. Photomicrograph.

EARLY STAGES OF DEVELOPMENT

The sperm itself could not be identified but the path followed in penetrating the egg could be seen readily (Pl. I, fig. 2). This figure also shows the island of cytoplasm carried into the yolk by



EXPLANATION OF PLATE III

1. Cross section through thoracic region of embryo at 10 hours to show middle plate differentiated. × 182, photomicrograph. 2. Cross section through head, thorax and abdomen of embryo at 13 hours to show further development of median plate and inner layer. × 177, photomicrograph.

the sperm. By 1½ hours the sperm nucleus was well defined in this cytoplasm and measured 8.5 μ in diameter.

At oviposition the egg nucleus is located in a thickened region of the periplasm at the anterior end of the egg, and in all of the eggs examined was in the metaphase or telophase of the first maturation division (text fig. 1).

Maturation of the egg nucleus is typical and is similar to that described by Rempel for *Mamestra* and Johannsen for *Diacrisia*. The first maturation division is completed in the first hour following oviposition; the second division by 1½ hours. The mature egg nucleus measured 6.8 μ in diameter. Two polar bodies resulted from the maturation divisions, one of which measured 8.5 μ in diameter, the other, 6.8 μ.

Formation of the cleavage cells and blastoderm is of the typical lepidopteran type and has been fully described for a phalaenid by Rempel. In *Heliothis*, 16 cleavage cells are present by 3 hours. By 4 hours they are approaching the periplasm with some remaining behind to form yolk cells (Pl. I, fig. 3). By 4½ hours the cleavage cells have entered the periplasm to form the blastema stage.

Cell membranes are developing between the nuclei in the periplasm at 5 hours, forming cuboidal blastoderm cells (Pl. I, fig. 4), but the membranes have not yet formed at the bases of the cells. Their inner surfaces are still very irregular so that the cytoplasm intermingles slightly with the yolk. The development of the cell membranes is more advanced in the equatorial region of the egg, indicating that the germ band cells differentiate slightly ahead of the serosa cells. An hour later the blastoderm cells are complete with the cell membranes formed on all sides. At this stage, the ends of the cells nearest the yolk are somewhat vacuolated. This may be due to the inclusion of some yolk material in the cytoplasm at completion of the cell membranes as described by Rempel.

FORMATION OF THE GERM BAND AND EMBRYONIC RUDIMENT

In a surface view of the egg the germ band first becomes distinguishable at about 6½ hours. At this time it has the form of a broad band encircling almost the entire equatorial periphery of the egg (Pl. II, fig. 1). It is oriented with its anterior margin, from which the head lobes will arise, toward the anterior, micropylar end of the egg. The lateral margins of the germ band are very close together, being separated by only a narrow strip of serosal cells. This narrow strip of cells widens out at the anterior and posterior poles of the egg and covers these regions completely which gives the serosa the "dumbell" shape described by Johannsen for *Diacrisia*.

The germ band now begins to grow narrower in a lateral direction so that its lateral margins gradually become farther apart. As this occurs, the strip of serosal cells between the lateral margins of the germ band broadens so that by 8 hours it has doubled its width. The serosal strip widens by a broadening and flattening of the existing cells rather than an increase in cell number by mitosis. As the germ band narrows, its consitituent cells also become narrower, changing from a cuboidal to a columnar form.

By 9 hours a distinct difference can be seen between the anterior and posterior ends of the germ band. The anterior end, near the micropyle, is narrowing more rapidly than the posterior end and the head lobes are beginning to appear. They are set off at this stage by the formation of shallow notches near the anterolateral margins of the germ band and by the formation of a median groove in the anterior end of the band (Pl. IV, fig. 1). This gives the anterior end of the band a bilobed appearance while the posterior end remains smoothly rounded. At the same time that the head lobes appear the germ band begins to elongate and extend over the anterior and posterior poles of the egg. By 11 hours the head lobes are well formed and the posterior end of the germ band has become distinctly cup shaped, covering the entire posterior pole of the egg.

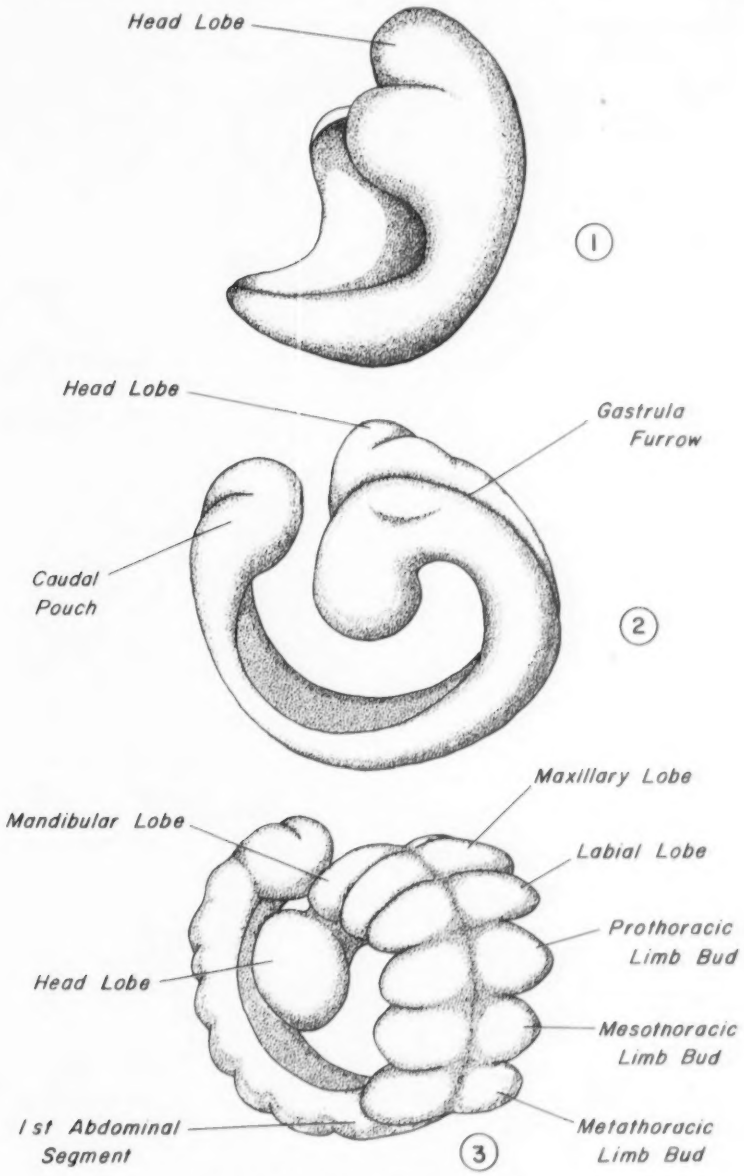
As with the Lepidoptera in general, the germ band is of the completely immersed type. The process of immersion begins in *Heliothis* at about 10 hours when the margins of the band curl over slightly and the anterior and posterior ends begin to sink into the yolk. These are pushed in more deeply as the germ band elongates and by 12 hours the germ band is completely surrounded by yolk.

As the germ band elongates, the region posterior to the head lobes becomes considerably narrower and by 12 hours the band is clearly differentiated into the protocephalon and protocorm. This condition is illustrated in Pl. IV, fig. 2. There is no external indication of any further subdivision of these regions. However, in longitudinal sections, the cells of the developing inner layer are segmentally grouped. Further differentiation begins an hour later when the thorax and the gnathal region of the head are divided into segments by the appearance of transverse grooves on the ventral surfaces. The formation of body segments proceeds posteriorly until by 16 hours the abdomen is clearly differentiated into 10 segments and a telson. The general appearance of the embryo during this period is illustrated in Pl. IV, fig. 3.

Concurrently with the development of segmentation in the head and thorax, six pairs of lobes appear ventrolaterally on these segments, indicating the start of the formation of the thoracic and gnathal appendages. The gnathal lobes are the largest at this time and constitute the developing mandibles, maxillae and labium.

At about this time, also, formation of the protocephalic appendages—the labrum and antennae—begins.

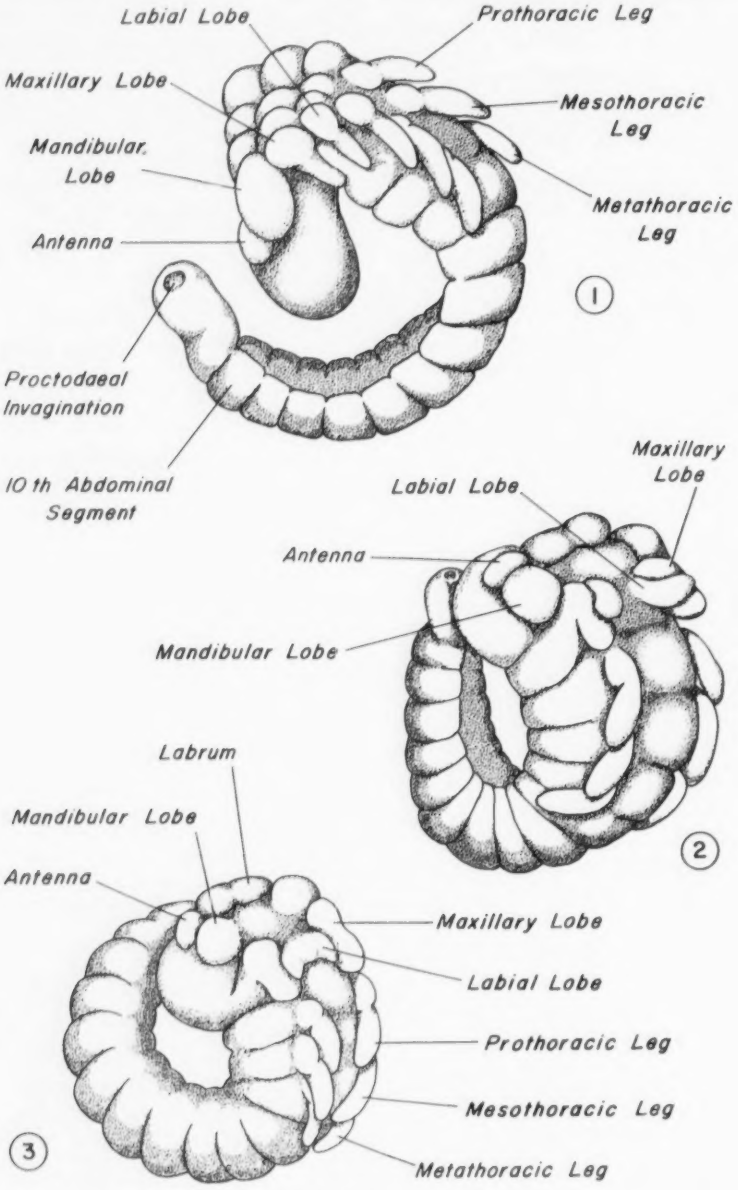
The labrum first appears as two widely separated lobes which arise on the inner dorsal margins of the head lobes (Pl. VI, fig. 1). At the same time the antennae arise on the lateroventral margins of the head lobes. By 19 hours both the antennal and labral lobes are more clearly defined. The stomodaeal invagination, which also forms at 16 hours, can be seen as a shallow depression in about the center of the ventral



EXPLANATION OF PLATE IV

1. Anterolateral view of embryo at 10 hours with yolk removed, showing head lobes forming. × 80. 2. Anterolateral view of embryo at 12 hours with yolk removed. × 80. 3. Ventrolateral view of embryo at 14 hours with yolk removed, showing gnathal and thoracic appendages forming. × 80.

surface of the head, between the lobes. The labral lobes gradually move closer together and meet at the center of the anterior margin of the head at about 28 hours (Pl. VI, figs. 2, 3). The labrum, when formed, thus lies just anterior to the stomodaeal opening. As development proceeds the labrum shifts its position slightly, moving ventrally and caudally between the head

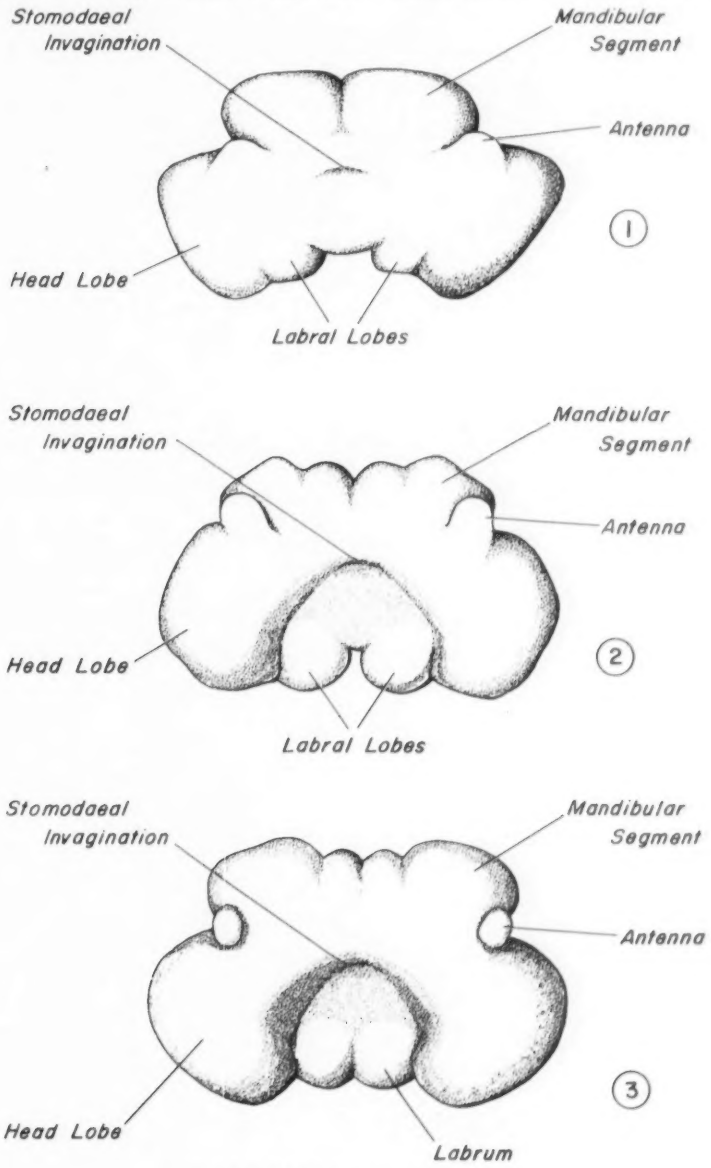


EXPLANATION OF PLATE V

1. Lateral view of embryo at 28 hours, with yolk removed, to illustrate general form and orientation of embryo at this time and further development of gnathal and thoracic appendages. × 125. 2. Ventrolateral view of embryo at 35 hours with yolk removed, showing close association of maxillary and labial lobes to begin formation of labial complex. × 114. 3. Ventrolateral view of embryo at 39 hours with yolk removed, showing further development of labial complex. × 96.

lobes so that it lies directly over the stomodaeal opening.

In lepidopteran larvae the hypopharynx, maxillae and labium are combined to form a compound structure. Development of this structure begins shortly after the stage shown in Pl. V, fig. 1 as the maxillary and labial segments become very closely associated. The labial appendages then alter their position and move medially toward the ventral mid-line of the embryo. At the same time each labial appendage comes into intimate contact with the maxillary appendage on the same side. This condition is illustrated in Pl. V, fig. 2. At 35 hours the labial



EXPLANATION OF PLATE VI

1. Anterior view of head of 19 hour embryo, showing antennal and labral lobes forming. × 156. 2. Anterior view of head of 24 hour embryo, to show further development of labral lobes. × 170. 3. Anterior view of head of 28 hour embryo, showing labral lobes fused to form labrum. × 198.

appendages are still separate and paired. By 41 hours, however, (Pl. V, fig. 3) the two have fused to form a single median lobe with the maxillary appendages forming closely associated lateral lobes. At about this same time the spinneret appears at the tip of the labial lobe. The development of the hypopharynx was not followed.

FORMATION OF THE EMBRYONIC MEMBRANES

Formation of the serosa and amnion in *Heliothis* follows the characteristic lepidopteran type described by Johannsen for *Diacrisia*. The two membranes form independently with the serosa completing its development well in advance of the amnion. Up to 6½ hours the serosal region remains directly continuous with the germ band (Pl. II, fig. 2). At this time the two become separated and the serosal region begins to expand, pushing over the outer surface of the germ band (Pl. II, fig. 2). The serosa spreads rapidly over the surface of the germ band and is completed at 9 hours. Many of the cells are two- or four-nucleate.

Formation of the amnion begins at the anterior and posterior ends of the germ band about an hour after the start of the serosa (Pl. II, fig. 4). The margins of the developing membrane push between the serosa and the outer surface of the germ band, working toward a central point from its anterior and posterior poles. The amnion is completed by 12 hours. It is thinner than the serosa and has uninucleate cells.

THE INNER LAYER

Eastham (1930), in his review on the formation of the germ layers in insects, lists three types of inner layer formation in the Lepidoptera; invagination of a middle plate, cell proliferation, and invagination from a gastrula furrow. A combination of these may occur in the same embryo as in *Heliothis* where both cell proliferation and middle plate formation take place.

Formation of the inner layer beings with the differentiation of a middle plate at about 10 hours. This extends through most of the length of the germ band, beginning between the head lobes and gradually disappearing toward the end of the abdomen. It is fairly narrow in the head region, but becomes wider and thinner in the thorax (Pl. III, fig. 1). Shortly after its differentiation the cells of the middle plate begin to proliferate and it assumes a wedge shaped form through the thorax and abdomen. At the same time, at about 11 hours, the middle plate begins to invaginate to form the inner layer. It gradually sinks below the level of the lateral plates, resulting in the formation of a groove on the ventral surface of the embryo. This constitutes the gastrula furrow and extends throughout the length of the embryo (Pl. II, fig. 2). As the middle plate sinks below the lateral plates they close over beneath it to form a complete ectoderm and an inner layer. After the invagination of the middle plate has been completed it begins to spread laterally and consequently thin out. This results in the formation of a typical inner layer, one cell thick, lying on the inner surface of the ectoderm. This condition is shown in Pl. II, fig. 2 where the inner layer has spread over the ectoderm in the thorax but still constitutes a wedge shaped mass of cells in the abdomen. Formation of the inner layer is completed by 16 hours. In the head region it is not well defined and consists of distinct, separate cells.

Differentiation of the inner layer follows two courses; the central region first breaks down into free cells throughout its length and the remaining lateral regions then form segmentally arranged pairs of coelomic sacs from which most of the other mesodermal structures are derived. Differentiation begins with the formation of the neural groove which appears first in the thorax at 16 hours, as illustrated in Pl. VII, fig. 1. The neural groove then elongates, gradually extending the length of the embryo, and also grows deeper.

The remainder of the inner layer, after formation of the free cells, constitutes the mesoderm and gives rise to the coelomic sacs. Formation of the coelomic sacs begins as the lateral margins of the germ band curl dorsally, and as they do so, carry the lateral margins of the mesoderm with them. The margins of the mesoderm then continue to curl over, forming sac-like structures with lumina in their centers (Pl. VII, fig. 3). A total of 15 pairs of coelomic sacs were identified in *Heliothis*. A pair of sacs were present in each of the 10 abdominal segments and the three thoracic segments. In addition, two pairs were identified in the head; one pair in the labial segment and a pair of poorly defined sacs in the labral lobes.

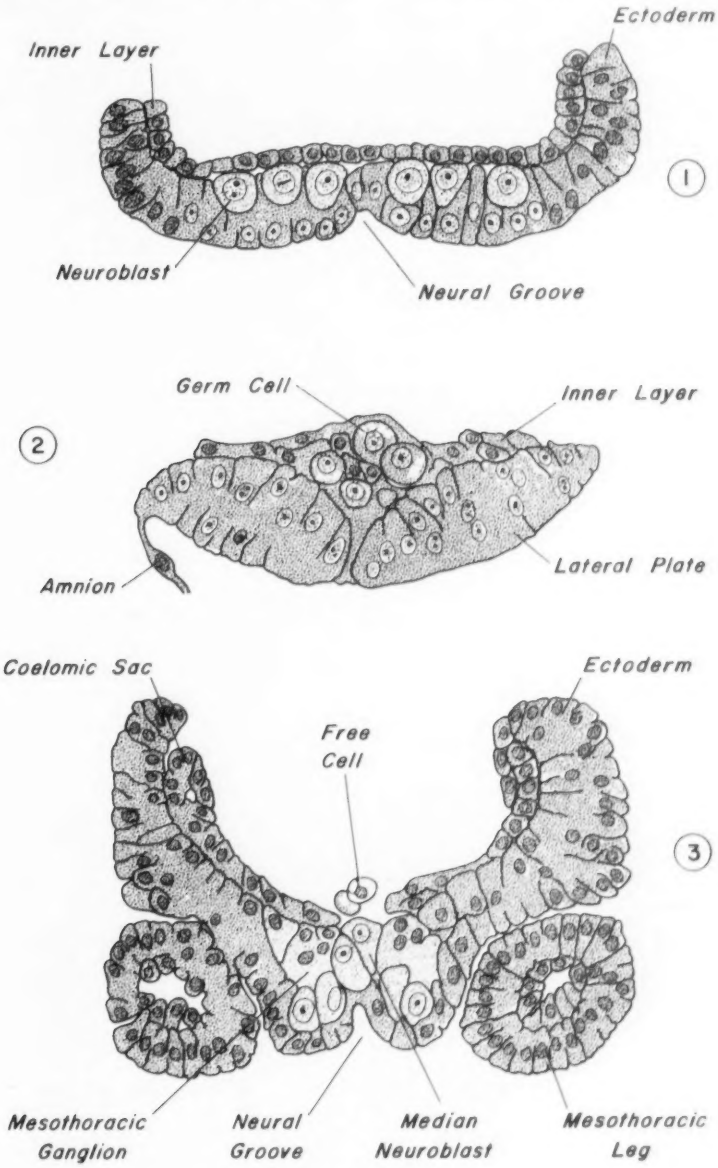
As the appendages grow out as sac-like evaginations of the body wall, a lining of inner layer cells is carried along with them. These provide a lining of mesoderm in each appendage. The tissue does not fill the appendage completely but retains a lumen in its center, as can be seen in a cross section (Pl. VII, fig. 3).

The cells forming the walls of the coelomic sacs become fairly large and columnar in shape. Ventrad to the sacs, some of the inner layer remains as a single layer of mesoderm cells lying against the ectoderm (Pl. IX, fig. 1). These cells are smaller and more irregular in form than those of the sacs. They give rise to the epithelial sheaths of the gonads and part of the somatic musculature. The coelomic sacs begin to break down at about 33 hours and this process is complete two hours later resulting in masses of mesoderm cells which form the musculature of the body wall and mid-gut.

Development of the blood cells in *Heliothis* could not be followed with certainty but they appear to arise as groups of cells just median to

the coelomic sacs. They are smaller than the free cells, averaging 3.4 μ in diameter. Based on their location in the embryo, they develop from some of the mesoderm cells which do not take part in the formation of the coelomic sacs.

An hour after the appearance of the neural groove the free cells begin to appear, lying separate from the inner surface of the ectoderm. Free cell formation is pronounced in the gnathal segments of the head by 19 hours. It then spreads throughout the rest of the embryo. The free cells, as they form, assume a characteristic



EXPLANATION OF PLATE VII

1. Cross section through thorax of embryo at 16 hours to show relation of neuroblasts to neural groove. × 538. 2. Cross section through abdomen of 16 hour embryo at the level of the germ cells. × 480. 3. Cross section through thorax of embryo at 22 hours, showing differentiation of inner layer and ganglion formation. × 552.

appearance. They have an irregular shape, usually with an eccentrically placed nucleus, and strongly vacuolated cytoplasm (Pl. VII, fig. 3). Some of these continue to increase in number by mitotic division.

Breakdown of the central region of the inner layer to form free cells is completed by 28 hours. By this time the cells average 6.8 μ in diameter and are no longer restricted to the embryo proper but are scattered throughout the egg among the yolk balls. They have become so vacuolated that the cytoplasm, except around the nuclei where it is thicker, appears mainly as a thin membrane holding the vacuoles together. The number of these cells was counted in two 28-hour-old embryos. In one, 766 were present; in the other 777. Eastham described many of these cells as giving rise to blood cells in *Pieris* as is the case in many insects. In *Heliothis*, however, these cells are the precursors of the fat body. They increase somewhat in size and begin to gather together in long highly vacuolated strands. This agglomeration of cells is completed by the time of hatching to form masses of fat tissue lying close to the body wall.

COMPLETION OF THE BODY WALL AND ASSUMPTION OF LARVAL FORM

During the early stages of development discussed so far and through about the first day and a quarter, the embryo assumes the form of a flat band with its margins curled dorsally (Pl. VII, fig. 1). Its ventral surface is covered with the amnion and dorsally it is open to the yolk. At this point, also, the embryo has reached a transitional stage of development. Histogenesis and the differentiation of the tissue layers is essentially complete and the conversion of the tissue systems into organ systems is beginning. It is during this period, at about 30 hours, that the completion of the body wall begins, transforming the embryo from the form of a flat band into that of a typical larva.

A provisional wall is first formed by a dorsal extension of the amnion and the lateral margins of the germ band. As these grow dorsally, a double wall structure results on either side. The outer wall is the amnion proper; the inner is the provisional body wall. The extensions from either side of the germ band meet and fuse along the dorsal mid-line resulting in a continuous body wall (Pl. XVI, fig. 2). In addition, after fusion, the amnion becomes continuous and completely surrounds the embryo. Completion of the body wall begins at the anterior and posterior ends of the embryo and proceeds toward the center. By 35 hours fusion along the dorsal mid-line has begun at the ends of the embryo and is complete throughout about 10 hours later.

In the head, as the amnionic folds approach each other and fusion takes place, the provisional body wall sinks in between the brain lobes, forming a shallow invagination. The walls of the invagination gradually grow together and fuse, forming a ridge-like projection extending below the head wall. A cuticle is secreted by the walls of this invagination concurrently with that over the rest of the body wall and constitutes the invaginated portion of the frons, a structure characteristic of lepidopteran larvae (Pl. IX, fig. 3).

The provisional body wall, as it forms, is almost as thin as the amnion. A few hours after its development begins, before dorsal closure is complete, the provisional wall begins to thicken and the cuboidal hypodermal cells of the permanent body wall arise. The thickening process begins ventrally and gradually extends dorsally so that the permanent body wall is completed almost simultaneously with completion of dorsal closure.

Secretion of the cuticle by the hypodermal cells begins at 59 hours. The cuticle appears first as a very thin, clear membrane around the mouth-parts and within a few hours has been formed around the entire body wall. It gradually increases in thickness until at hatching it measures 1.2 μ thick except in the head where it is somewhat thicker. Also, in the head region, about 10 hours before hatching, the cuticle develops a dark pigmentation to form the "black head" stage frequently mentioned in the literature.

BLASTOKINESIS

During its early development and the period of completion of the body wall, as it assumes the form of a larva, the embryo undergoes a number of changes in position in the egg, all of which can be included under the general term "blastokinesis." In *Heliothis*, the germ band, after its differentiation, continues to increase in length with the head and tail pushing more deeply into the yolk until it reaches a maximum length at about 16 hours. During this period and up to about 28 hours the embryo maintains the same position in the yolk (text fig. 2A) with the anterior end near the micropyle. At this time it begins to undergo a rotation through 90° so that at 33 hours it lies wrapped around the equator of the egg (text fig. 2B). At the same time it shortens in length so that the tail no longer overlaps the head. The embryo then undergoes another change in orientation to assume the coiled form characteristic of a *Heliothis* larva at hatching. In order to assume this configuration, from its position wrapped around the equator, the head and thorax curve back upward with the head finally coming to lie directly below the micropyle (text fig. 2C).

The embryo undergoes a final rotation in which the body turns through 180° along its longitudinal axis. This constitutes the major blastokinetic movement of the embryo and reverses its position with respect to the yolk. The ventral surface now faces inward, toward the yolk; the dorsal surface faces outward toward

the chorion. This rotation does not occur simultaneously throughout the entire embryo but begins with the abdomen at about 42 hours and in three hours gradually works forward until the entire embryo has completed its revolution.

After the completion of rotation and assumption of the coiled form illustrated in text fig. 2C the embryo does not change its position again until hatching. At this time (82 hours) the fully developed larva chews through the chorion and crawls out of the egg. It then consumes the remainder of the chorion and embryonic membranes.

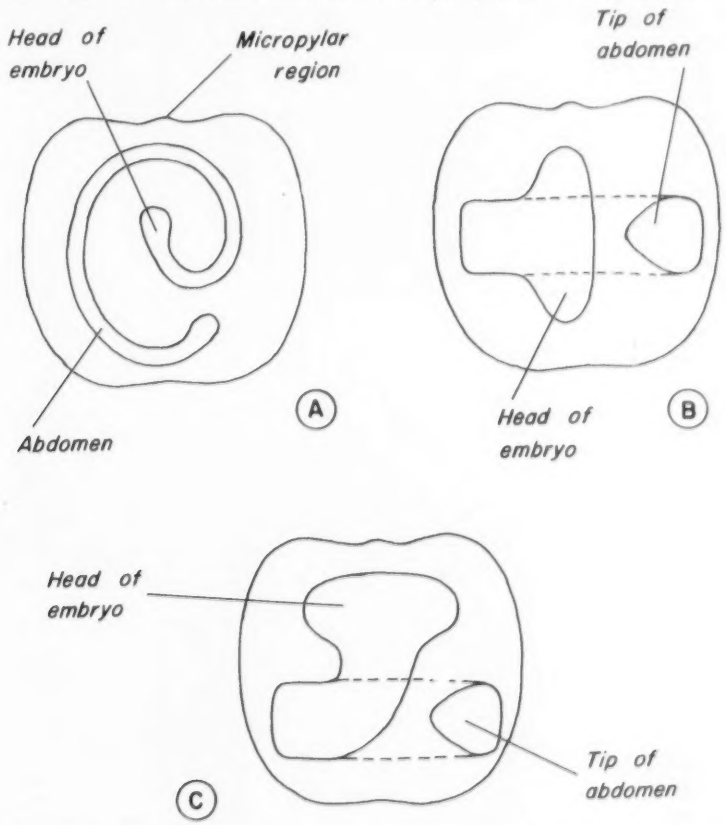
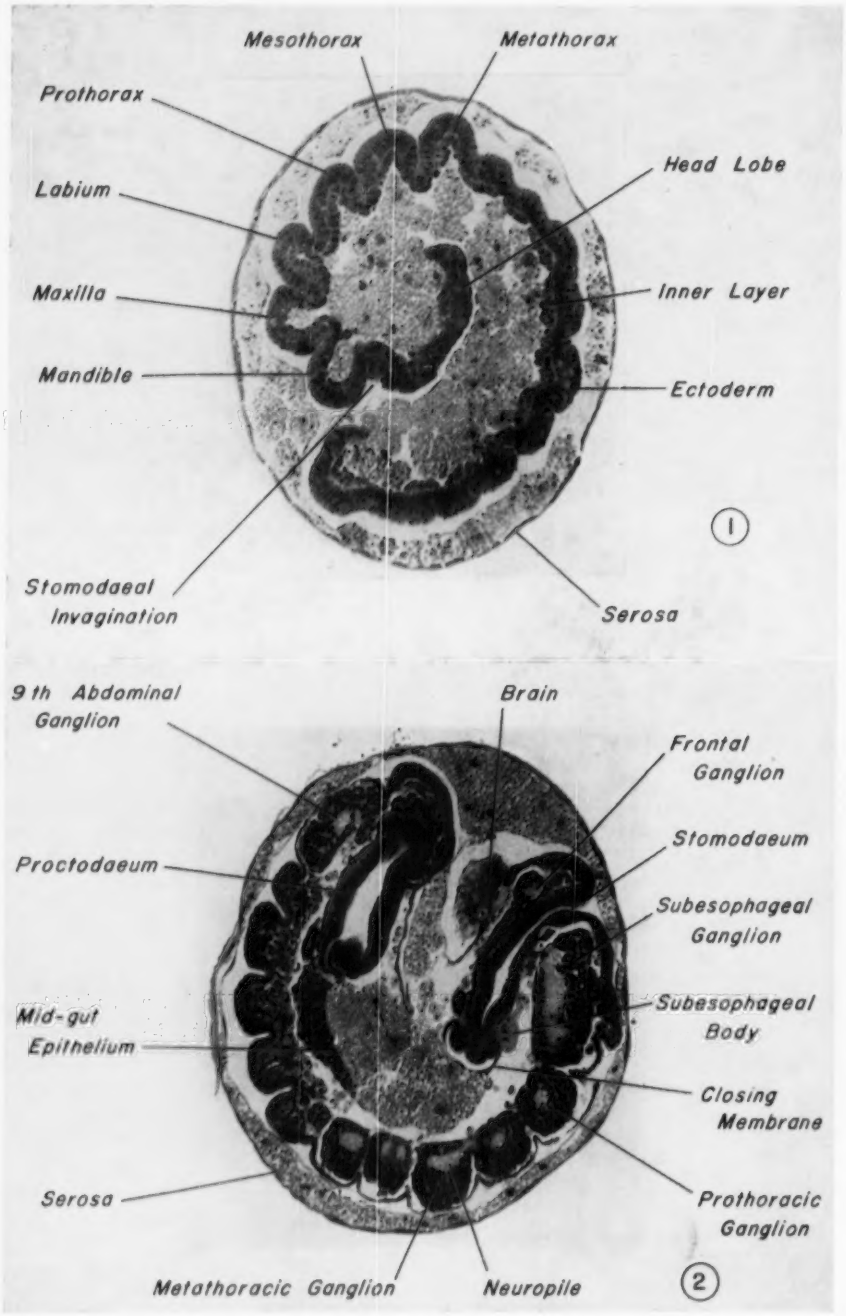


FIG. 2. A, diagrammatic lateral view of egg to illustrate orientation of embryo at 16 hours. B, diagrammatic lateral view of egg to illustrate orientation of embryo at 33 hours; embryo has rotated through 90° from position illustrated in A. C, diagrammatic lateral view of egg to illustrate orientation of embryo at 48 hours; rotation and closure of body wall completed.

It is interesting to consider the influence which the shape of the egg exerts on the orientation and movements of the embryo. In the flat, disk shaped egg studied by workers such as Huie (1918) on *Eudemis* and Rutschky (1949) on *Grapholitha* the embryo is necessarily confined to a "two dimensional" form and its major blastokinetic movement—that which reverses the relation of the dorsal and ventral surfaces to the yolk—is accomplished by the "tail whipping" method (Rutschky 1949). In the hemispherical egg of *Catocala*, studied by Hirschler (1905), the embryo has a little more room for movement and in its early development begins to assume the spiral form illustrated for *Heliothis* in Pl. II, fig. 1 although in *Catocala* the head never overlaps the tail. In a spherical egg such as that of *Heliothis* or of *Diacrisia* the embryo has about equal room available in any direction for development and the spiral form becomes quite pronounced as the embryo grows in length. Both *Catocala* and *Heliothis* undergo the 90° rotation indicated in text fig. 2A but subsequent to this *Heliothis* is able to attain a greater degree of coiling (text fig. 2C).

Eudemis also undergoes a comparable 90° rotation but it does so at a much earlier stage of development, when the germ band is still straight and has not assumed a curved form. Huie suggested that this first rotation in *Eudemis* is necessary to permit development of the appendages.

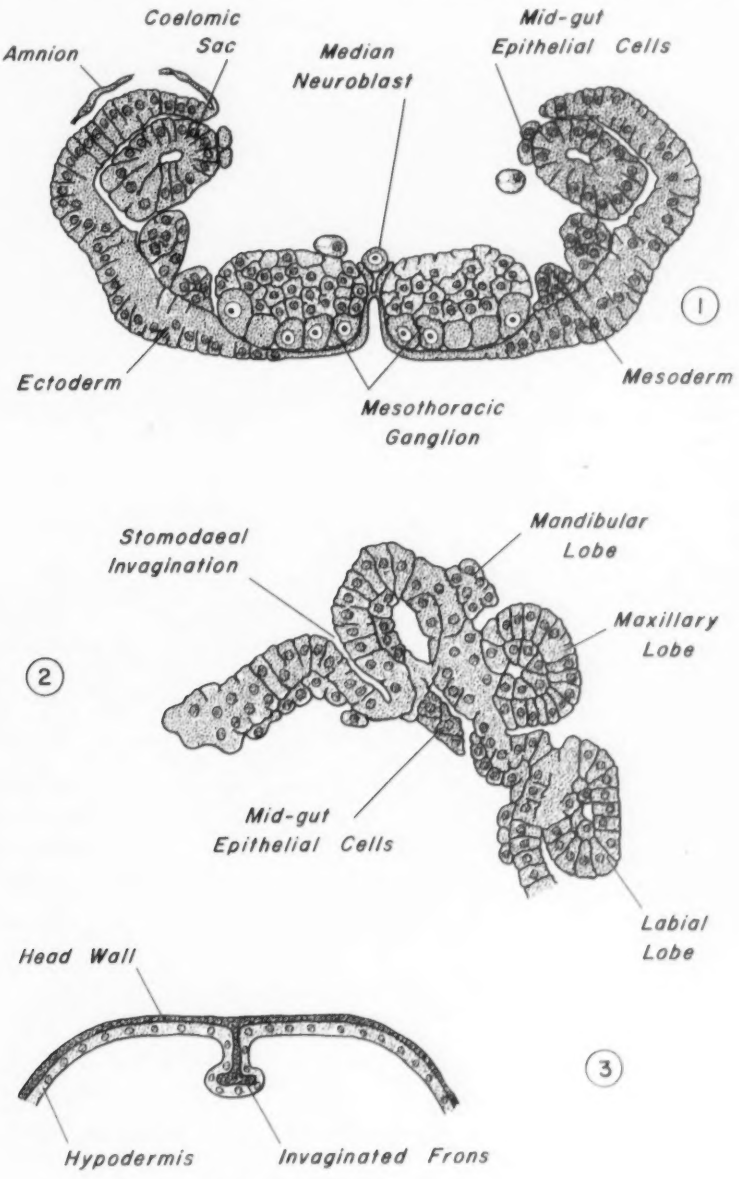
It has been postulated by some workers that



EXPLANATION OF PLATE VIII

1. Sagittal section through embryo at 16 hours. × 160, photomicrograph. 2. Sagittal section through embryo at 37 hours. × 190, photomicrograph.

the movements of embryos in eggs are due to the necessity for the embryo to move away from its waste products or to an area where the food material has not been depleted. This may be true in those insects where the embryo is comparatively small with respect to the size of the egg. However, in the Lepidoptera, where the eggs themselves are rather small, the author is inclined to agree with Tirelli, (after Hagan 1951) who believed that at least some of the movements



EXPLANATION OF PLATE IX

1. Cross section through mesothorax of embryo at 28 hours, showing coelomic sacs and ganglion development. × 500. 2. Longitudinal section through head region of embryo at 25 hours, shows relation of mid-gut epithelial cells to stomodaeal invagination. × 398. 3. Cross section through dorsal head wall of embryo at 81 hours to show invaginated frons. × 475.

were of a mechanical nature to utilize the space available for development to the best advantage.

ALIMENTARY CANAL

Development of the alimentary canal begins with the formation of the stomodaeum and proctodaeum. The stomodaeum appears first, at 16 hours, as a shallow invagination between the head lobes. This can be seen in longitudinal section in Pl. VIII, fig. 1 and in surface view, a few hours later, in Pl. VI, fig. 1. The proctodaeal invagination appears about an hour after the stomodaeum near the tip of the abdomen. The tip of the abdomen first doubles over, folding back upon itself, and the invagination appears in the folded over portion. The invaginations gradually elongate, the proctodaeum developing more rapidly and becoming longer than the stomodaeum. As the proctodaeum develops it lies parallel to the long axis of the abdomen but the stomodaeum pushes in at an angle to the body. The cells forming the walls of the two regions remain columnar so that the walls are fairly thick. However, the cells at the inner end of the stomodaeum become very thin, forming a closing membrane. The margins of the membrane recurve around the end of the stomodaeum so that the structure assumes a mushroom like shape (Pl. XI, fig. 2). The closing membrane is well formed by 28 hours. As the invaginations develop they carry in with them a lining of mesoderm cells from the inner layer. These cells eventually differentiate to form the muscular linings of the fore and hind gut.

The development of the mid-gut is very typical and is similar to that described in such species as *Diacrisia* (Johannsen, 1929) and *Pieris* (Eastham, 1930b). It forms from paired anterior and posterior epithelial strands, arising from cell masses beneath the stomodaeal and proctodaeal invaginations. The anterior mass was first identified at 25 hours and consisted of a short strand of cells projecting back from the inner end of the stomodaeal invagination (Pl. IX, fig. 2). The posterior group of entoderm cells was identified an hour later lying at the distal end of the proctodaeum. As the cells in each group multiply in number they separate into a pair of ribbons. The paired anterior and posterior ribbons increase in length, growing toward one another, and become continuous near the center of the embryo at 31 hours. They then consist of two lateral strands of entoderm cells lying just above the coelomic sacs. These can be seen in cross section in Pl. IX, fig. 1. The strands become wider as the cells increase in number. At about 40 hours they meet along the ventral mid-line forming a broad, trough-like plate of cells. The lateral margins then grow dorsally and meet along the dorsal mid-line to form the tubular mid-gut which encloses all of the yolk remaining in the embryo. The closure along the dorsal mid-line is completed at 48 hours, most of the growth having taken place during the period of rotation. The mid-gut cells, just before closure is completed, show no indication of differentiation but retain the same appearance as those of the mid-gut ribbons.

Shortly after the mid-gut begins to grow dorsally, the splanchnic mesoderm masses begin to spread and follow the developing mid-gut walls. By the time of completion of the mid-gut wall the mesoderm surrounds the gut with a thin sheath of cells which will form the mid-gut musculature. The splanchnic mesoderm lies just below the mid-gut ribbons.

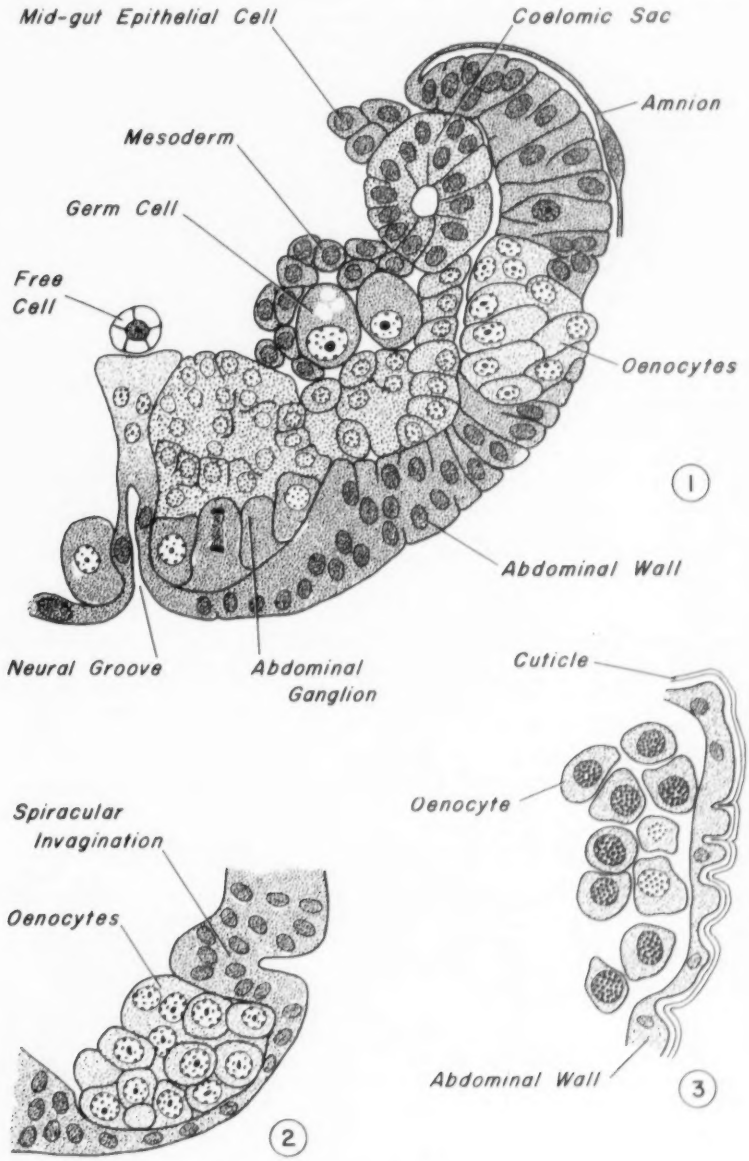
The first indication of the differentiation of the mid-gut cells occurs 5 or 6 hours after completion of the gut wall. At this time the cells begin to elongate. By 60 hours they are definitely columnar in form although the cytoplasm still stains uniformly and the nuclei are located at the center of the cells. A few vacuoles have appeared at their distal ends. Ten hours before hatching the distal halves of the cells are highly vacuolated and the yolk in the gut has become very homogeneous in nature; the yolk balls have completely broken down. The vacuoles and nature of the yolk indicate that the mid-gut cells are now secreting digestive enzymes. A few hours later a distinct brush border appears. Within a few hours of hatching special cells, termed goblet cells, appear, interspersed throughout the mid-gut epithelium. These have been described in the mid-gut of a number of insects; particularly in larval Lepidoptera (Wigglesworth, 1947). The nuclei of these cells are located in the basal region whereas in the other mid-gut cells the nuclei are located in the middle. The distal two thirds of each of these special cells consist of a long, tubular, duct-like invagination of the cell membrane. This duct-like region is continuous with the gut lumen and is lined with a brush border.

THE MALPIGHIAN TUBULES

The Malpighian tubules arise at the inner end of the proctodaeal invagination as six tubular invaginations of the wall of the proctodaeum. There is no indication that they are endodermal in origin as was reported by Henson (1932) for *Pieris brassicae*. They appear at 26 hours and are arranged laterally in two groups of three each, one group on either side of the proctodaeum (Pl. XVI, fig. 1). The tubules grow posteriorly, elongating rapidly so that they measure 60 μ in length only two hours after their appearance. The walls of the tubules are very thick and in cross section are composed of five or six wedge-shaped cells (Pl. XIII, fig. 2), each containing a large, dark staining nucleus. The lumina of the tubules are quite small.

In lepidopteran larvae, the Malpighian tubules do not empty directly into the proctodaeum. Instead, the three tubules on each side open first

into an ampulla which in turn opens into the proctodaeum. This arrangement is illustrated for the larvae of *Vanessa* by Henson (1937). The tubules in the embryo of *Heliothis* arise at first as direct evaginations of the proctodaeum while the ampullae do not appear until several hours later. The ampullae arise at the point where each group of three tubules on each side fuse with each other to form a short common duct. This region then expands and becomes



EXPLANATION OF PLATE X

1. Cross section through right side of fifth abdominal segment of 29 hour embryo, showing developing oenocytes and relation of germ cells to mesoderm. × 1070. 2. cross section through segment of right side of abdominal wall of 36 hour embryo to show oenocytes fully invaginated. Note relation of oenocytes to spiracular invagination. × 1070. 3. Cross section through segment of right side of abdominal wall of 60 hour embryo. Note oenocyte nuclei now filled with granular material. × 1070.

thin walled to form the sac-like ampulla. The third tubule on each side does not empty directly into the ampulla but joins one of the other tubules just distad to the ampulla. A cross section through the Malpighian tubules and developing ampullae is shown in Pl. XVI, fig. 2.

As the tubules develop they become enveloped in a nucleated sheath which forms the larval musculature. The nuclei of the sheath cells are ellipsoidal in form with their long axes at right angles to the long axis of the tubules. Generally one to several of these nuclei may be seen in a cross section of a tubule (Pl. XIII, fig. 2). The sheath arises from the mesodermal cells carried in as a layer around the proctodaeal invagination. Its development lags behind that of the tubules, not appearing until several hours after them. Some of the mesoderm cells then push out along the tubules to form the sheath. When they first appear, the cells of the sheath are fairly thick but they gradually become thinner so that the nuclei appear as prominent bulges. After the ampullae develop, the sheath becomes restricted to these structures and cannot be located along the tubules themselves. This is to be expected since Palm (1946), in his study of the musculature of Malpighian tubules, listed the Lepidoptera as having musculature only around the basal region.

No striated border or protoplasmic processes develop in the tubules during embryonic life. In the later stages the tubule walls consist, in cross section, of only two or three cells instead of five or six as in the early stages. This indicates that at least part of the increase in length of the tubules must be accounted for by a cellular rearrangement rather than by cellular division.

THE SUBESOPHAGEAL BODY

The subesophageal body first appears at 26 hours as a group of cells lying along the ventral wall of the stomodaeal invagination. Their cytoplasm is uniform and light staining. A longitudinal section through these cells at a later stage is shown in Pl. VIII, fig. 2. Shortly after the subesophageal cells appear, their cytoplasm becomes filled with many small vacuoles and this character persists throughout the remainder of their development.

At first the cells rapidly increase in number forming a broad band. Within a few hours this separates into two large lateral masses as indicated in Pl. XV, fig. 2. The subesophageal body reaches its maximum size by 35 hours, then gradually decreases in size. It persists throughout embryonic development and is still present at the time of hatching.

THE SILK GLANDS

Lepidopteran silk glands, which are modified labial salivary glands, arise in *Heliothis* as a pair of widely separated invaginations of the body wall between the labial and prothoracic segments. A similar situation has been described for *Diacrisia* by Johannsen (1929). These invaginations appear at 26 hours and shortly thereafter shift anteriorly so that they are definitely associated with the posterior region of the labial segment. As the appendages of the labial segment begin to move together to form the labium, the two invaginations also approach each other and finally join at their proximal ends to a common duct for the silk glands. The common duct then passes into the spinneret as it forms at the distal end of the labium at 41 hours. The silk glands elongate very rapidly, finally extending through the length of the abdomen. In cross section they consist of two or three cells and are thick walled with a small lumen. About 12 hours before hatching the walls of the glands become much thinner as the diameters of the lumina increase. The lumina then quickly become filled with a homogeneous, even staining secretion which probably forms the silk when extruded.

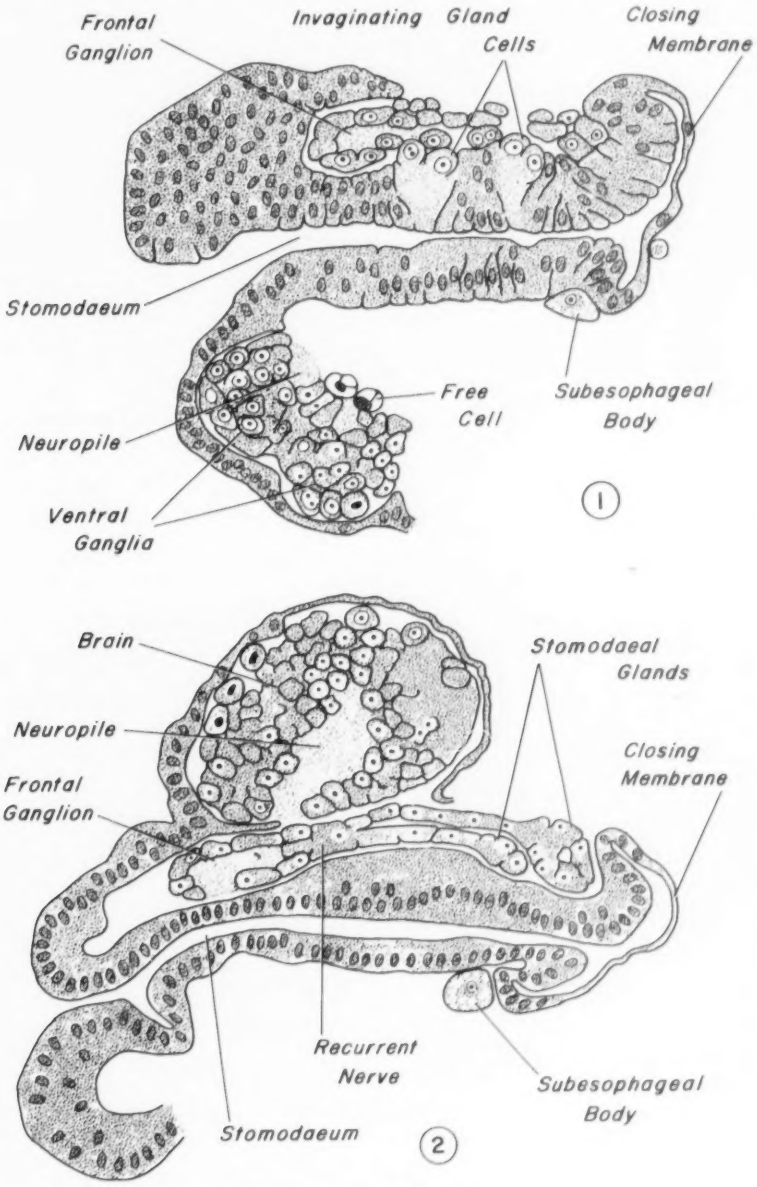
THE HEART

Formation of the heart has been described in detail by Eastham for *Pieris*. In *Heliothis* the cardioblasts could first be distinguished at 36 to 37 hours in the typical position along the dorsal margins of the somatic mesoderm, well before completion of the mid-gut or body wall. As they form, they become triangular in shape with the base of the triangle facing dorsally. The two basal corners are drawn out into thin membranous extensions. On the outer side the membranous extension attaches to the body wall at the point where the temporary wall is becoming thicker to form the permanent wall. The extension of the inner corner of the cardioblast attaches to the splanchnic mesoderm below the mid-gut strand. The cardioblasts are moved dorsally through these two points of attachment. As the thickening of the temporary body wall to form the permanent wall develops dorsally, the point of attachment of the cardioblast is carried along with it. In like manner, the inner corner of the cardioblast is carried along with the splanchnic mesoderm as the latter grows dorsally with the developing mid-gut wall. The resulting pull on the cardioblasts causes them to become somewhat attenuated and assume their characteristic crescent shape (Johannsen and Butt 1941). This condition is illustrated for *Heliothis* in Pl. XIII, fig. 1. A few free cells are pushed ahead of the cardioblasts and are enclosed in the heart as it forms.

Fusion of the cardioblasts to form the heart begins in the posterior region of the abdomen and gradually progresses anteriorly. Fusion occurs first along the dorsal margins of the cells, next to the body wall, at 44 hours as illustrated in Pl. XIII, fig. 1 and is followed by fusion of the ventral margins of cells. Closure of the heart is completed by 46 hours.

THE RESPIRATORY SYSTEM

Formation of the respiratory system begins in the thorax at 26 hours. At this time paired invaginations begin to form in the body wall directly opposite the coelomic sacs. These gradually deepen to form the tubular spiracular invaginations. Within two hours they have formed in the abdominal segments also (Pl. XVI, fig. 2). At 40 hours three branches develop from the inner end of each invagination. One runs



EXPLANATION OF PLATE XI

1. Longitudinal section through stomodaeum of 30 hour embryo to show development of stomatogastric nervous system. × 702. 2. Longitudinal section through brain and stomodaeum of 36 hour embryo, composite drawing from several sections to show relations of brain and components of stomatogastric system. × 468.

dorsally to the developing somatic musculature and a second runs ventrally to the segmental ganglion. The third develops anteriorly toward the spiracular invagination in the preceeding body segment. A longitudinal tracheal trunk is thus formed on each side of the body by 42 hours, joining all the spiracles together. The intima which forms the tracheal lining appears at 60 hours, at about the same time as the appearance of the cuticle over the rest of the body. When the spiracular invaginations first form they are thick walled and show many nuclei in cross section. However, as the tracheae form and elongate, the walls become thinner and show only one or two nuclei in cross section. Thus much of the tracheal development is due to a cellular rearrangement rather than to increase in cell number.

THE OENOCYTES

The oenocytes develop as groups of cells beneath the spiracular invaginations in the first 8 abdominal segments. They arise from modified ectoderm cells at 29 hours. At this time a group of cells in the body wall, ventrad to each spiracular invagination, becomes lighter staining. This condition is illustrated in Pl. X, fig. 1, although the spiracular invagination did not show on the section illustrated. Each of these pockets of cells then pushes in to form an invaginated, densely packed mass and the body wall closes over beneath them (Pl. X, fig. 2). Verson (after Stendell, 1912) also reported that the oenocytes arose from a group of ectoderm cells while Stendell (1912) reported that each segmental group of oenocytes arose from a single ectoderm cell. The body wall remains much thinner directly opposite the developing oenocytes so that for a number of hours the latter appear to be imbedded directly in the wall. The cells are completely invaginated within two hours after their initial appearance. As the entire body wall grows thinner, the oenocytes become completely separated from it and also separate slightly from each other. Throughout embryonic development they remain as loosely aggregated clusters of cells close to the body wall in the region of the spiracles (Pl. X, fig. 3). The oenocytes were never observed in division and presumably the number present at hatching is the same as the original number of modified ectoderm cells although Stendell reported that in *Ephestia* they increase by amitotic division.

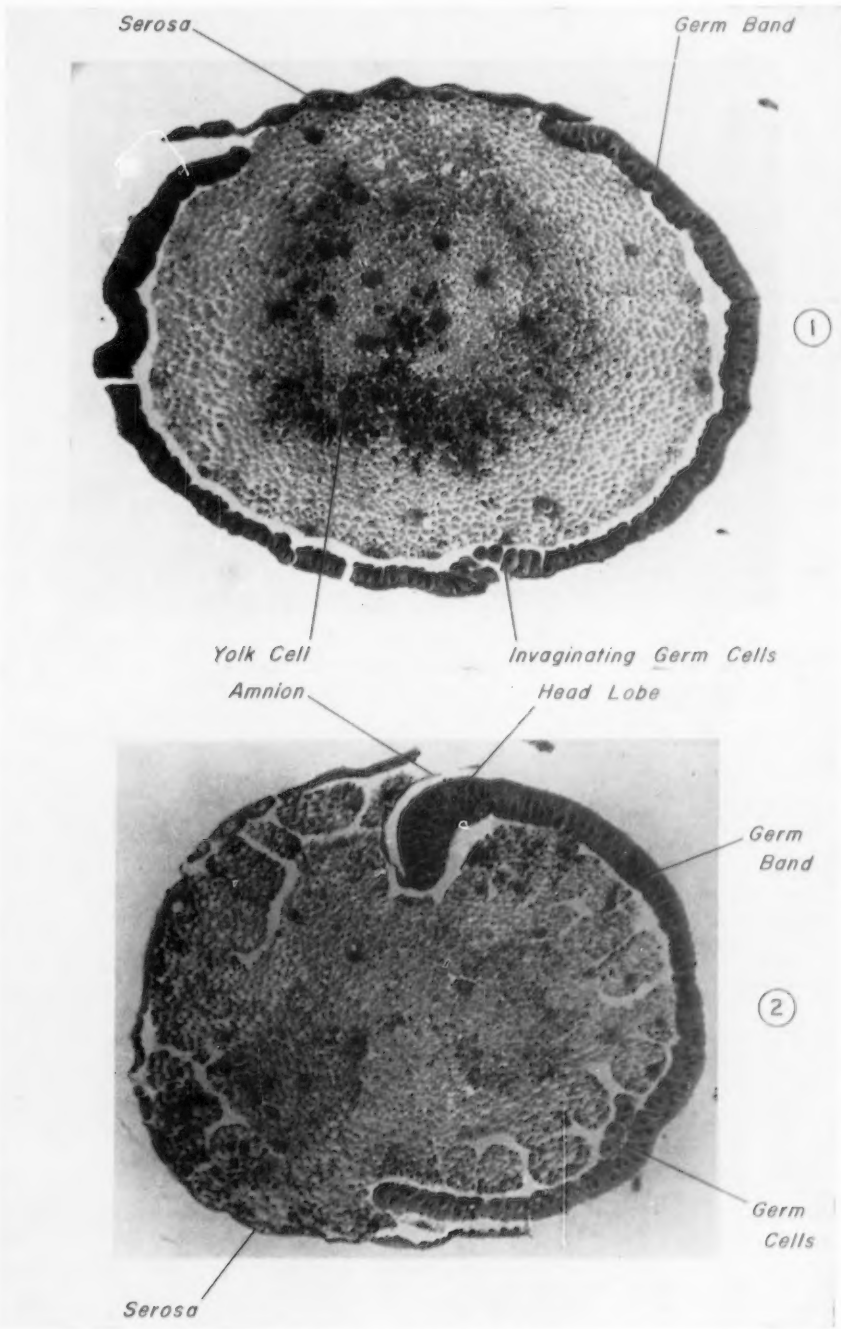
The cytoplasm of the oenocytes is very uniform, even staining and non-granular in character and the cells measure 6 to 8 μ in diameter. Their nuclei pass through a definite cycle during development which indicates secretory activity and can be correlated in time with the formation of the cuticle. When the oenocytes first form, the nuclei are spherical or somewhat oval in shape with a prominent, dark staining, central nucleolus. The chromatin is rather sparse and granular in nature as indicated in Pl. X, fig. 1. Most of it is localized around the periphery of the nuclei with a lesser amount scattered throughout. By about 56 hours, shortly before the start of the formation of the cuticle, the character of the nuclei changes. They become densely packed with very dark staining granules which appear identical with chromatin granules. These are so numerous as to virtually obscure the nucleoli, which are still present. The amount of granular material present is at a maximum from 60 to 70 hours. By 73 hours the nuclei begin to return to their original condition and by 76 hours the nucleoli are again prominent with rather sparse chromatin material. During this second change in the character of the nuclei the oenocytes themselves increase in size and become oval in shape. At the time of hatching they range from 8 to 14 μ in length.

Stendell also reported a definite cycle of secretory activity for the embryonic oenocytes in *Ephestia* although he observed the appearance of clear, non-staining inclusions in the nuclei which later passed into the cytoplasm. A number of authors (Wolf, 1954) have correlated oenocyte activity with new cuticle formation during moulting. The coincidence of cuticle formation with nuclear changes in the oenocytes in *Heliothis* embryos indicated that they are serving a similar function here.

THE NERVOUS SYSTEM

Formation of the central nervous system begins with the appearance of the neuroblasts. In *Heliothis*, the neuroblasts first appear at 13 hours, before the completion of the inner layer or the development of the neural groove. In contrast to many other structures, they do not appear first in the thorax but are spread throughout the length of the germ band, lacking only in the head lobes where they do not appear until two hours later. When the neuroblasts first arise they are rather widely separated from one another in a lateral direction with six to eight usually appearing in a cross section. The cells are fairly large, globular in shape and lie along the ventral surface of the germ band. A fairly constant character is the presence of one to several apical vacuoles. The cytoplasm at this stage stains slightly darker than that of the surrounding ectoderm cells, although later this character becomes reversed.

By 16 hours the neural groove is forming in the thoracic region and the neuroblasts have moved away from the ventral surface of the germ band, now lying along the inner surface. This condition is illustrated in Pl. VII, fig. 1. The cytoplasm has also become lighter staining than that of the surrounding ectoderm cells. Also, at this time, the neuroblasts in the thorax have become arranged into two lateral groups, one on



EXPLANATION OF PLATE XII

1. Cross section through germ band at 8 hours, shows germ cells invaginating and yolk cells concentrated in region of "Auflösungszone". × 208, photomicrograph. 2. Sagittal section through embryo at 10 hours, showing germ cells completely invaginated. × 212, photomicrograph.

either side of the neural groove. In cross section the neuroblasts in each group can be seen to be arranged in a row of three (Pl. VII, fig. 1).

The median neuroblasts arise at 17 hours along the inner margin of the neural groove. These are shown in cross section, at 22 hours, in Pl. VII, fig. 3. Eastham has described a similar development for the median neuroblasts in *Pieris* although he suggests that in *Pieris* they give rise to the transverse connectives in the segmental ganglia, which is not the case in *Heliothis*. In this species we believe they form the median nerve. The neural groove gradually elongates so that a few hours after the start of the stomodaeal and proctodaeal invaginations it extends the length of the embryo. By the time the neural groove has completed its elongation the neuroblasts begin to divide mitotically, producing chains of daughter cells which will form the nerve cells.

Ganglion formation begins as the intersegmental grooves deepen, dividing the neuroblasts into segmental, paired masses. Further development of the ganglia in the ventral nerve cord occurs as the chains of daughter cells produced by repeated division of the neuroblasts gradually become longer. The neural groove continues to increase in depth so that by 26 hours each ventral ganglion consists of two distinct lateral cell masses in each segment (Pl. IX, fig. 1). A longitudinal section at this stage clearly shows segmental masses of ganglion cells in the 3 gnathal segments, the 3 thoracic segments and 10 abdominal segments.

The neural groove is lined with two walls of ectoderm and as the ectodermal invagination pushes deeper, it forms a pocket around the median neuroblast so that this cell is completely separated from the rest of the ganglion (Pl. IX, fig. 1). At 28 hours the neuropile begins to appear along the dorsal margins of the lateral cell masses in the ganglia and an hour later the two lateral halves of each ganglion are joined together by the appearance of the cross commissures. The cross commissures in each ganglion develop anteriorly to the median neuroblasts. By this time each median neuroblast has undergone several divisions to give rise to a short column of cells. At this time also, the ectodermal walls of the neural groove begin to fuse and the single wall so formed gradually shrinks and disintegrates. The lateral halves of each ganglion can thus come into intimate contact and the characteristic ganglionic form develops. This process of fusion is completed by 37 hours and the neuropile has become covered dorsally by more ganglion cells. The median neuroblast cells remain as a distinct group in the median dorsal region of each ganglion. This stage of development can be seen, at 42 hours, in Pl. XV, fig. 3.

Shortly after the appearance of the cross commissures the neuropile begins to elongate in a longitudinal direction and the series of ganglia are joined together at 31 hours by the development of the longitudinal connectives. The lateral nerves begin to grow out from the thoracic ganglia a few hours after the appearance of the longitudinal connectives. Two of these develop on either side. One pair arises from the lateromedian region of the ganglion and the other pair from the laterodorsal region. The latter pair grow somewhat dorsally toward the region of the spiracular invaginations. The former pair grow toward, and become intimately associated with, the mesodermal cells which are destined to form part of the musculature of the body wall.

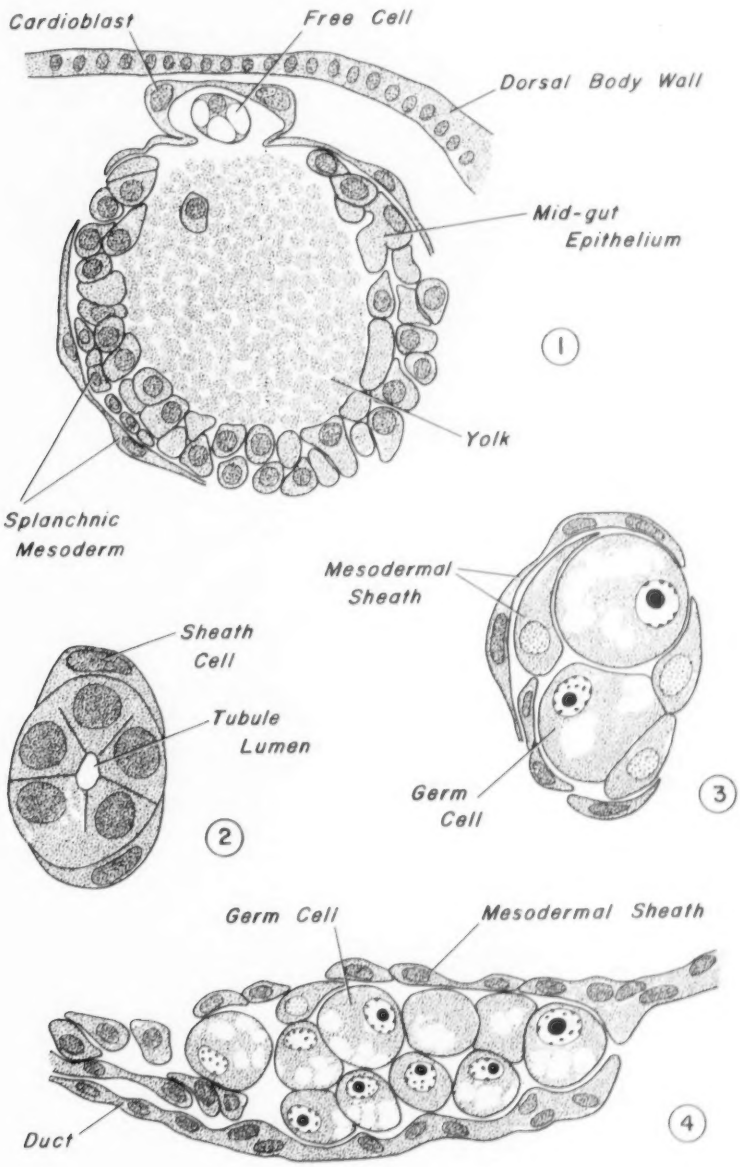
Shortly after the appearance of the lateral nerves a branch from the tracheal invagination develops ventrally and enters the ganglion along the path of a lateral nerve, extending into the neuropile. This can be seen in Pl. XV, fig. 3.

The neurilemma appears as a very thin membrane around the ganglia and along the lateral nerves several hours after the entrance of the tracheae into the ganglia. On the basis of the slides we believe it is formed by a flattening of some of the peripheral, ganglion cells. This method is the same as that described by Roonwall (1937) in the Orthoptera. There was no indication that it is formed from mesoderm of the median neuroblasts as has been suggested by some authors.

As the gnathal ganglia begin to form they arise as three distinct structures. However, shortly after the appearance of the neuropile in them, they become completely confluent to form the subesophageal ganglion. This process is completed by 35 hours and the resulting subesophageal ganglion can be seen in Pl. VIII, fig. 2.

A similar process occurs in the eighth, ninth, and tenth abdominal ganglia. These arise as distinct ganglia although the tenth is never very large. The ninth and tenth become fused first and a short time later combine with the eighth. The three are fully fused by 39 hours to form the compound eighth abdominal ganglion.

The neuroblasts begin to appear in the head lobes at 15 hours and increase rapidly in number during the next few hours. They then begin to bud off columns of ganglion cells and each head lobe is completely filled with cells by 30 hours, although the two groups are not in contact with each other. Very shortly the neuropile begins to develop mesad from each lobe just anterior to the stomodaeum. The neuropile becomes continuous between the brain lobes at 35 hours. At no time during the development of the brain are individual ganglia present. The earliest indication of segmentation in this structure is the lobular form of the neuropile which can be seen in longitudinal section in Pl. XVII, fig. 2, indicating the proto-, deuto- and tritocerebral areas of the brain. However, even these subdivisions of the neuropile arise secondarily from a primary,

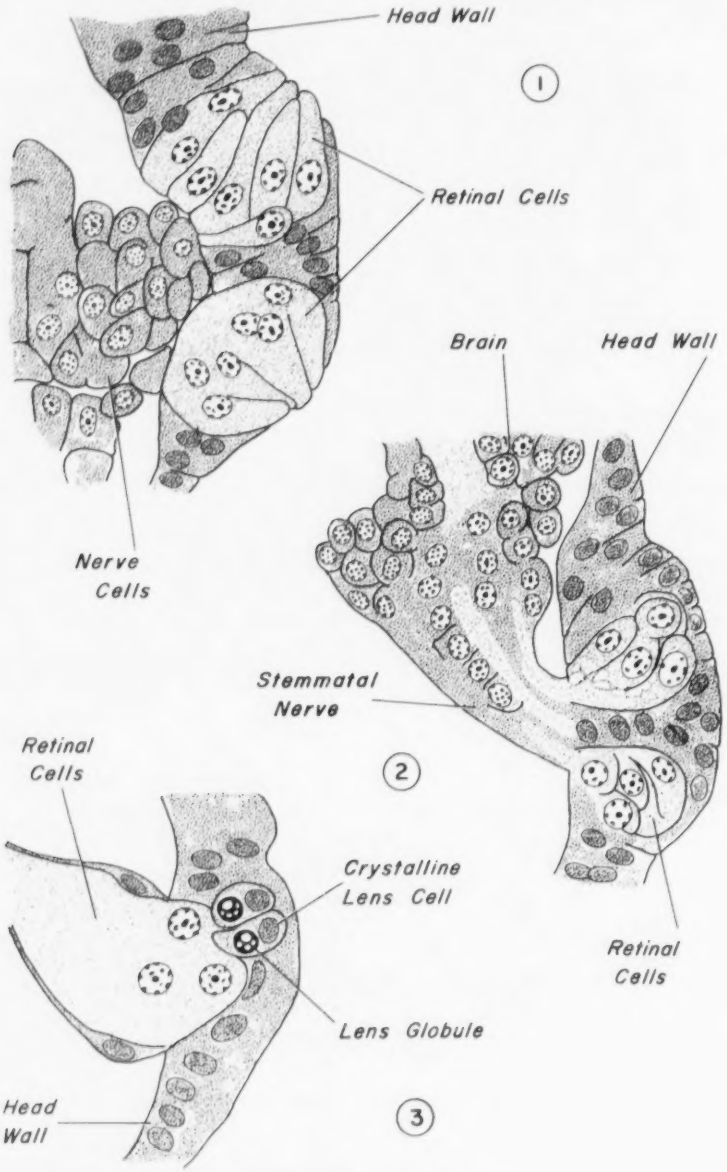


EXPLANATION OF PLATE XIII

1. Cross section through developing mid-gut and heart in abdomen of 44 hour embryo. Note heart already closed along its dorsal wall but still open along its ventral wall. × 937. 2. Cross section through Malpighian tubule at 37 hours. Note mesodermal sheath cells surrounding tubule. × 3080. 3. Cross section through developing gonad at 40 hours to show relation of mesodermal sheaths to germ cells. Note difference in cells of inner and outer sheaths. × 1685. 4. Longitudinal section through developing gonad at 37 hours. × 1400.

uniform neuropile region. The tritocerebrum lies in the anterior ventral region of the brain with the proto- and deutocerebral areas behind it. At about the same time that the neuropile regions become well defined, the brain separates from the ectoderm and thus lies free of the developing body wall.

The circumesophageal commissures grow out from the brain concurrently with the appearance of the longitudinal connectives in the ventral



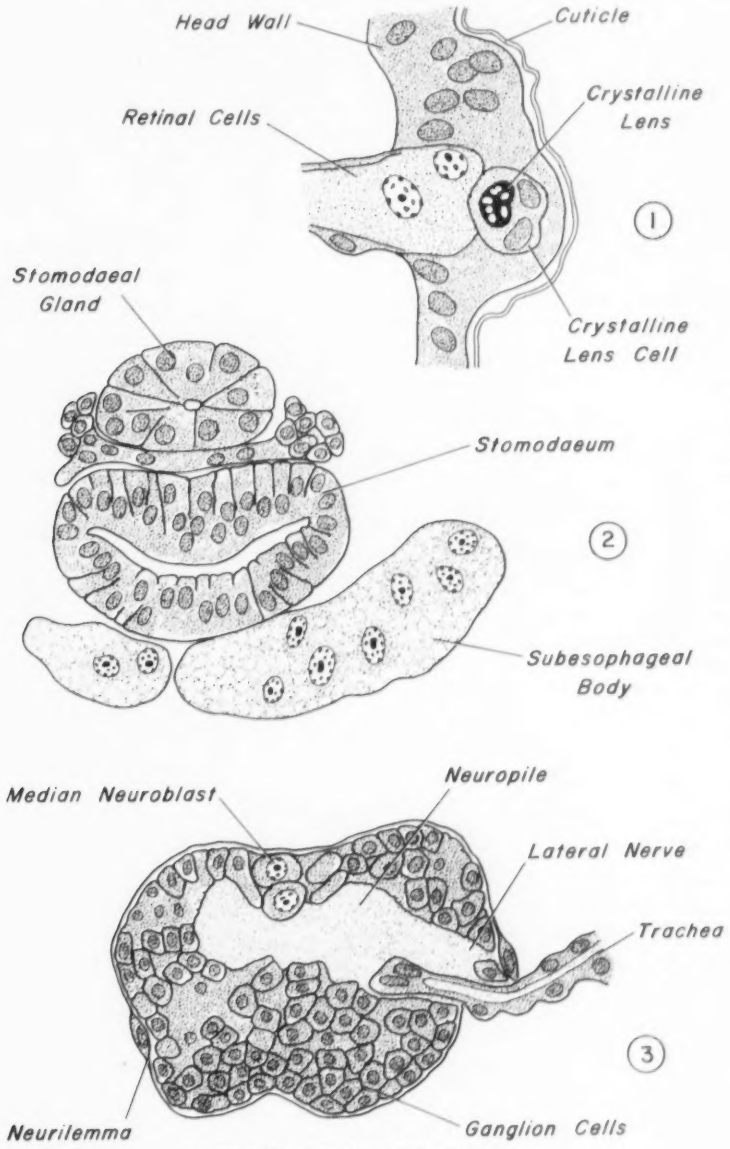
EXPLANATION OF PLATE XIV

1. Cross section through lateral head wall of 30 hour embryo in region of retinal cells. × 1380. 2. Cross section through lateral head wall of 36 hour embryo in region of retinal cells, showing stemmatal nerve developing between brain and retinal cells. × 1130. 3. Cross section through lateral head wall of 56 hour embryo in region of retinal cells; retinal cells fused, development of crystalline lens beginning. × 1369.

nerve cord. They join with the subesophageal ganglion at 33 hours. A cross section through the tritocerebral area of the brain at this time is shown in Pl. XVII, fig. 1.

The development of the frontal ganglion, recurrent nerve and two associated glandular structures was followed in *Heliothis*. No indication was found of the presence of occipital ganglia.

The frontal ganglion begins to develop at 28 hours when a few cells invaginate from the proximal dorsal wall of the stomadaeum, just posterior to the developing labrum. The cells invaginate from two points in the wall, one



EXPLANATION OF PLATE XV

1. Cross section through lateral head wall of 60 hour embryo in region of stemmata, showing further development of crystalline lens. × 1420. 2. Cross section through distal end of stomodaeum at 36 hours to show stomodaeal gland and subesophageal body. × 1000. 3. Cross section of abdominal ganglion at 42 hours. Note entrance of tracheae into neuropile region along lateral nerve. × 715.

directly behind the other. These cells multiply in number and within two hours a neuropile begins to appear in the mass. The recurrent nerve then grows out from the frontal ganglion and extends posteriorly along the dorsal wall of the stomodaeum. This is illustrated in Pl. XI, fig. 1. Eastham describes a similar formation of the recurrent nerve in *Pieris*, terming it the stomatogastric nerve. As the head develops, the frontal ganglion shifts forward, coming to lie well in front of the brain as indicated in longitudinal section in Pl. XVII, fig. 2.

Two glandular structures, referred to here as stomodaeal glands, arise at the distal end of the stomodaeum in similar fashion to the frontal ganglion. Each gland is formed from a separate group of cells which invaginate from the dorsal stomodaeal wall. The cells from which the glands arise have large nuclei similar in structure to those of neuroblasts and appear shortly after the start of development of the frontal ganglion. The invaginating cells are illustrated in Pl. XI, fig. 1. The glands become widely separated from the frontal ganglion as the stomodaeum elongates but remain associated with the ganglion through the recurrent nerve. The nerve comes into contact with the glands at 35 hours as it extends posteriorly along the stomodaeal wall. Pl. XI, fig. 2 illustrates this stage of development. The glands reach maximum size at 36 hours. The posterior one is the larger of the two, measuring 26 μ in diameter, and shows a distinct lumen. A cross section through this structure is shown in Pl. XV, fig. 2. Shortly after they reach maximum size the two glands fuse, gradually decrease in size, and disappear while the embryo is undergoing rotation. In view of their origin and location, the glands may represent the corpora cardiaca. However, except for contact with the recurrent nerve, they contain no nerve tissue, as is usually described in the corpora cardiaca, and they cannot be located in older embryos.

THE STEMMATA

In *Heliothis* larvae six stemmata or lateral ocelli are present in a semi-circle on either side of the head. Structurally each stemma consists of a number of elements: the cornea, corneagen cells, crystalline lens, crystalline lens-forming cells, and the retina. The retina constitutes the light sensitive unit and is continuous with the stemmatal nerve.

The stemmata are ectodermal derivatives and develop from modified cells in the head wall. Their development in *Heliothis* is similar to that described by Busselman (1934) in *Ephestia kuhniella*. In *Heliothis* they begin to form at 30 hours with the development of the retina. At this time, at the locus of each stemma, a group of seven ectodermal cells become modified to form retinal cells. Each such group will form the retina of a mature stemma. Each ectodermal cell, in forming a retinal cell, first becomes lighter staining than the surrounding tissue (Pl. XIV, fig. 1). The distal ends of these cells then become broader and rounded, assuming a club shaped appearance, and project above the surface of the head wall in a characteristic bulge (Pl. XIV, fig. 2). The nuclei at this time are located in the broad distal ends of the developing retinal cells and are characterized by larger nucleoli and less chromatin material than those of the surrounding ectodermal cells.

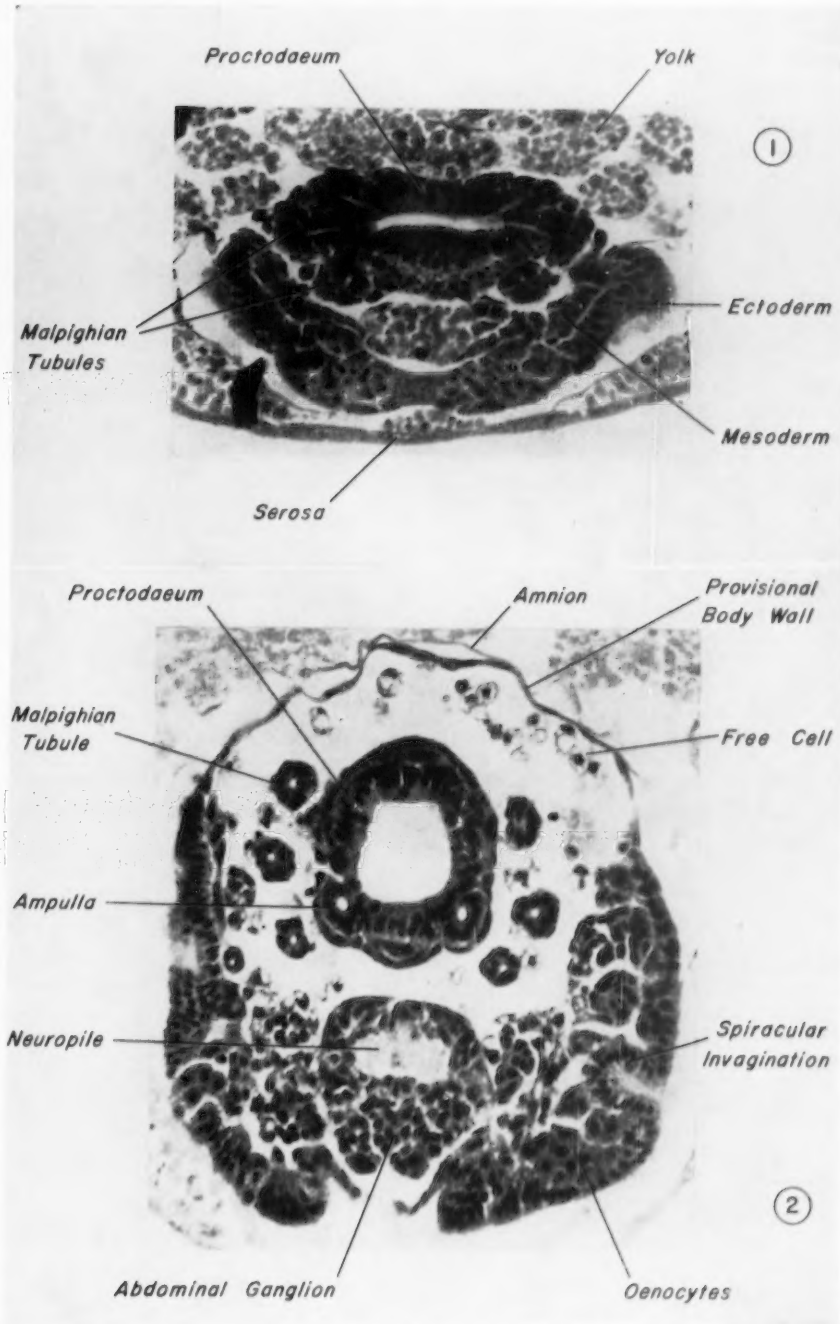
At the same time that the retinal cells first appear the bases of the nerve cells of the developing brain are closely appressed to the head wall (Pl. XIV, Fig. 1). The stemmatal nerve develops from these nerve cells and by 33 hours the retinal cells are directly continuous with it (Pl. XIV, fig. 2).

As development proceeds, the retinal cells increase considerably in size while the head wall becomes thinner. The cells thus project well into the head cavity and become surrounded by a nucleated sheath (Pl. XIV, fig. 3). The seven retinal cells of each stemma become indistinguishably fused and their cytoplasm becomes highly vacuolated. About 12 hours before hatching the rhabdome appears in the center of each retinal group.

A few hours after the retinal cells appear, some of the surrounding ectodermal cells push over them so that the head wall becomes continuous above them (Pl. XIV, fig. 2). It is from these ectodermal cells that the cornea and crystalline lens of each stemma are formed.

The crystalline lens is a tripartite structure formed as a secretion product of three crystalline lens-forming cells. These cells arise from normal ectodermal cells at 46 hours. They can be distinguished by their larger nuclei which are spherical rather than elliptical in shape. Shortly after their differentiation dark staining globules begin to accumulate in the cytoplasm of the cells. Two of these cells are illustrated at this stage in Pl. XIV, fig. 3. This material gradually builds up in amount until each cell is almost filled with a dark staining mass. The materials in each of the three cells become so closely associated as to constitute a single mass forming the crystalline lens as illustrated in Pl. XV, fig. 1. The lens remains surrounded, however, by the lens-forming cells. Scattered throughout the crystalline lens are a number of clear, non-staining vacuolated areas (Pl. XIV, fig. 3; Pl. XV, fig. 1).

Several additional ectodermal cells around the periphery of each group of crystalline lens cells become specialized as corneagen cells and secrete the cornea. This is formed concurrently with the rest of the cuticle.



EXPLANATION OF PLATE XVI

1. Cross section through inner end of proctodaeum at 28 hours to show invagination of Malpighian tubules. × 475, photomicrograph. 2. Cross section through abdomen of 40 hour embryo in region of proctodaeum. Note ampullae appressed to proctodaeal wall. × 545, photomicrograph.

THE REPRODUCTIVE STRUCTURES

The insects in which the time of differentiation of the germ cells has been determined have been divided into five groups by Nelson (1934). *Heliothis* would fall into his third grouping along with the other Lepidoptera, *Endromis*, *Ephestia* and *Solenobia*. In this third group the germ cells appear shortly after the germ band is differentiated but before the formation of the inner layer. In *Heliothis* this occurs at about 8 hours, at which time a small area of cells in the center of the germ band, near the mid-line, become more rounded and spherical in form. The nuclei enlarge somewhat and the cells push in toward the yolk to form a shallow invagination (Pl. XII, fig. 1). The region of invaginated cells thus formed measures 60 μ in length. Within an hour the edges of the invagination close over beneath it, resulting in a mass of cells lying just within the germ band. A longitudinal section of the embryo after invagination of the germ cells is shown in Pl. XII, fig. 2. The cells gradually become almost spherical in shape. Their nuclei become much larger than those of the germ band cells, being more similar to the nuclei of the yolk cells from which they may be distinguished by their more finely granular, lighter staining chromatin. Each nucleus has one or two very large, black-staining nucleoli. A group of 25 germ cells was counted in a 9-hour embryo, after their invagination was completed. The germ cells will eventually separate into two lateral groups in order to form the gonads. For some time, however, they remain in the mid-line of the embryo, becoming surrounded by the middle plate cells as the latter sink in to form the inner layer (Pl. VII, fig. 2). Separation of the germ cells into lateral groups appears to be passive. As the inner layer spreads laterally, after invagination, the germ cells are carried along. By 21 hours they constitute two widely separated groups, localized in the fifth and sixth abdominal segments where they lie ventrad and somewhat median to the coelomic sacs (Pl. X, fig. 1). When the germ cells separate into lateral groups they do so in approximately equal numbers. This is borne out by counts of germ cells at several stages of development. For example, at 28 hours 8 cells were counted in one group and 9 in the other. At 44 hours 9 cells were counted in one and 10 in the other. At 59 hours, one group had 8, the other 10. In addition, the germ cells do not increase in number during embryonic development as is indicated by the above counts.

Formation of the gonads begins shortly after separation of the germ cells into lateral groups as the latter becomes loosely surrounded with mesoderm cells (Pl. X, fig. 1). These cells will give rise to the epithelial sheaths of the gonads and are derived from the mesoderm masses which lie ventrad to the coelomic sacs. Few, if any, of the cells come from the coelomic sacs themselves. As the gonads develop, they become more compact in form and assume an oval shape in cross section, the germ cells forming a central core surrounded by a sheath of mesoderm (Pl. XIII, fig. 3). As shown in Pl. XIII, fig. 4, they are elongated longitudinally. The mesoderm tissue extends posteriorly beyond the gonad to form the duct.

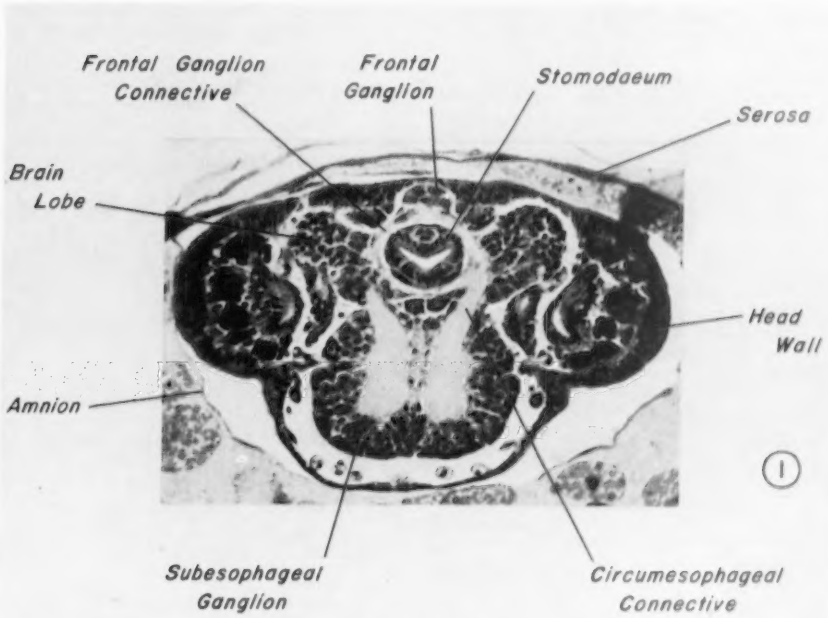
The mesoderm surrounding the germ cells becomes arranged in two layers, forming an inner and an outer sheath. When these first form, at 40 hours, the cells of the inner sheath are larger, with lighter staining nuclei (Pl. XIII, fig. 3). These become wedged-shaped, pushing between the germ cells around the periphery of the gonad. The smaller cells of the outer sheath form a continuous layer of tissue around the entire gonad.

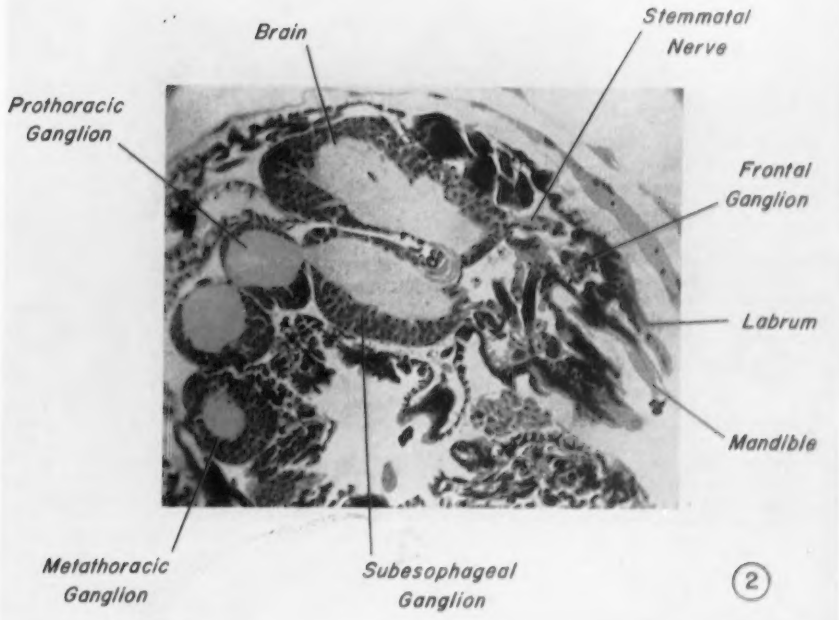
At 30 hours, as the gonads are forming, they become arranged laterally between the lateral margins of the abdominal wall and the splanchnic mesoderm beneath the mid-gut ribbons. As the body wall develops dorsally the gonads are carried along. At about the time the mid-gut is completed the gonads reach their definitive position, lying close together in the fifth abdominal segment above the wall of the mid-gut.

At this time, the embryonic development of the gonads is essentially completed; they undergo no further change except that the mesodermal sheaths become thinner.

CHRONOLOGICAL DEVELOPMENT OF THE EMBRYO OF HELIOTHIS ZEA (BODDIE) AT 25°C.

0–15 minutes	Egg nucleus in first maturation division.
1 hour	Egg nucleus in second maturation division.
1½ hours	Mature egg nucleus in yolk, close to sperm. Two polar bodies in periplasm.
3 hours	Cleavage nuclei in 16 cell stage.
4½–5 hours	Cleavage nuclei enter the periplasm to form the blastema.
6 hours	Blastoderm cells complete.
6–6½ hours	Differentiation of blastoderm into germ band and serosa.
8 hours	Formation of amnion begins. Germ cells appear.
9 hours	Serosa completed. Head lobes appear at the anterior end of the germ band. Invagination of the germ cells completed.
10 hours	Formation of the middle plate begins.
11 hours	Proliferation of cells from the middle plate begins. Gastrula furrow appears as invagination of the middle plate.
12 hours	Germ band differentiated into protocorm and protocephalon. Amnion completed.
13 hours	Segmentation begins. Thoracic and gnathal appendages appear. Neuroblasts appear.
15 hours	Neuroblasts appear in the head lobes.
16 hours	Segmentation completed. Stomodaeal invagination begins. Formation of the inner layer completed. Differentiation of the inner layer begins. Neural groove appears. Germ band reaches maximum length.
17 hours	Median neuroblasts appear. Free cells appear. Proctodaeal invagination begins.





EXPLANATION OF PLATE XVII

1. Cross section through head of 44 hour embryo. × 315, photomicrograph. 2. Longitudinal section through head of 70 hour embryo to illustrate relationships of components of central nervous system. × 245, photomicrograph.

21 hours	Formation of coelomic sacs begins. Separation of germ cells into lateral groups completed.
25 hours	Anterior mid-gut strands begin formation.
26 hours	Malpighian tubules appear. Posterior mid-gut strands begin formation. Silk glands invaginations appear. Subesophageal body appears. Spiracular invaginations appear.
28 hours	Labral lobes fuse. Breakdown of the central region of the inner layer is completed. Neuropile begins to appear. Formation of the frontal ganglion begins.
28–30 hours	Germ band rotates through 90° and lies wrapped around the equator of the egg.
29 hours	Oenocytes arise. Cross commissures arise in the nerve ganglia. Formation of stomodeal glands begins.
30 hours	Retinal cells arise. Formation of the labial complex begins. Formation of the gonads begins. Completion of body wall begins.
31 hours	Ventral ganglia joined longitudinally by connectives. Anterior and posterior mid-gut ribbons become continuous. Invagination of the oenocytes is completed.
33–35 hours	Coelomic sacs break down to form somatic and splanchnic mesoderm.
35 hours	Fusion of gnathal ganglia to form subesophageal ganglion is completed. Closure of body wall begins. Neuropile becomes continuous between brain lobes. Recurrent nerve contacts stomodaeal glands.
36–37 hours	Cardioblasts appear. Stomodaeal glands reach maximum size.
39 hours	Fusion of the eighth, ninth and tenth abdominal ganglia is completed.
40 hours	Epithelial sheaths of the gonads developing. Lateral mid-gut strands join along mid-line to form broad plate of cells. Tracheal branches arise from the inner ends of the spiracular invaginations.
41 hours	Labial complex completed.
42 hours	Longitudinal tracheal trunks formed.
42–45 hours	Embryo rotates through 180° along its longitudinal axis.
44 hours	Fusion of the cardioblasts begins along their dorsal margins to form the heart.
45 hours	Dorsal closure and body wall completed.
46 hours	Closure of the heart is completed. Crystalline lens forming cells begin to differentiate.
48 hours	Mid-gut wall completed.
56 hours	Dark staining granules appear in oenocyte nuclei.
59 hours	Formation of the cuticle begins.
60 hours	Intimal lining appears in tracheae. Mid-gut cells become columnar in form.
60–70 hours	Maximum amount of granular material present in oenocyte nuclei.
72 hours	Lumina of silk glands become larger in diameter and filled with a homogeneous secretion. Pigment is deposited in the head wall to form the "black head" stage. Mid-gut cells become vacuolated and the yolk changes in character indicating that the mid-gut has assumed a secretory activity.
73–76 hours	Oenocytes increase in size and the nuclei return to their original condition.
82 hours	Embryo emerges from the egg and consumes the chorion and embryonic membranes.

TIME-TABLE OF STRUCTURAL DEVELOPMENT OF THE EMBRYO OF HELIOTHIS ZEA (BODDIE) AT 25° C.

Maturation of the egg

0–15 minutes	Egg nucleus in first maturation division.
1 hour	Egg nucleus in second maturation division.
1½ hours	Maturation of egg nucleus completed; two polar bodies present.

Formation of the blastoderm

2 hours	First cleavage nuclei appear.
3 hours	Cleavage nuclei in 16 cell stage.
4½–5 hours	Cleavage nuclei enter the periplasm to form the blastema.
6 hours	Blastoderm cells completed.

Formation of the germ band and embryonic rudiment

6½ hours	Germ band and serosa differentiated in surface view. Germ band begins to grow longer and narrower.
9 hours	Anterior end of germ band becomes bilobed as head lobes appear.
10 hours	Germ band begins to sink into the yolk.
12 hours	Germ band completely immersed and differentiated into protocephalon and protocorm.
13 hours	Segmentation begins as transverse grooves appear on the ventral surface.
16 hours	Segmentation completed.

The appendages

13 hours	Thoracic and gnathal appendages appear as lobes on ventral surface of the germ band. Labral and antennal lobes appear.
28 hours	Labral lobes meet and fuse.
30 hours	Maxillary and labial lobes become closely associated to begin formation of the labial complex.
41 hours	Labial lobes fuse; spinneret appears at tip of resulting labium.

The embryonic membranes

6 hours	Serosa cells differentiated at completion of blastoderm.
6½ hours	Serosa separates from germ band and begins to expand.
9 hours	Serosa completed.
8 hours	Formation of amnion begins.
12 hours	Amnion completed.

Formation of the inner layer.

10 hours	Middle plate appears.
11 hours	Invagination of the middle plate begins.
16 hours	Formation of the inner layer is completed.

Differentiation of the inner layer

16 hours	Differentiation begins with the appearance of the neural groove.
17 hours	Free cells appear as breakdown of the central region of the inner layer begins.
28 hours	Breakdown of the central region of the inner layer is completed.
21 hours	Formation of the coelomic sacs begins.
33–35 hours	Coelomic sacs break down.

Completion of the body wall.

30 hours	Dorsal extension of the amnion and lateral margins of the germ band to form the provisional body wall begins.
35 hours	Closure of the provisional body wall begins at the anterior and posterior ends of the embryo.
45 hours	Closure of the body wall is completed.
59 hours	Secretion of the cuticle by the hypodermal cells begins.

72 hours	Pigment is deposited in the head wall to form the "black head" stage.

Blastokinesis

16 hours	Embryo reaches maximum length.
28–33 hours	Embryo rotates through 90° to lie wrapped around the equator of the egg.
42–45 hours	Embryo rotates through 180° along its longitudinal axis.

The alimentary canal

16 hours	Stomodaeal invagination appears.
17 hours	Proctodaeal invagination appears.
25–26 hours	Mid-gut epithelial cells arise at inner ends of stomodeal and proctodaeal invaginations.
31 hours	Anterior and posterior mid-gut ribbons become continuous.
40 hours	Mid-gut epithelium expands dorsally.
48 hours	Dorsal closure of mid-gut completed.
60 hours	Mid-gut cells become columnar in form.
72 hours	Mid-gut cells become highly vacuolated.

The Malpighian tubules

26 hours	Malpighian tubules arise as invaginations of the proctodaeal wall.
30 hours	Mesodermal sheath develops around tubules.

The silk glands

26 hours	Silk gland invaginations first appear.
41 hours	Common duct extends into spinneret as it forms.
70 hours	Glands become filled with a homogeneous, even staining secretion.

The heart

36–37 hours	Cardioblasts first appear.
44 hours	Closure of the heart begins.
46 hours	Closure of the heart is completed.

The oenocytes

29 hours	Oenocytes first appear as modified ectoderm cells.
31 hours	Invagination of the oenocytes is completed.
56 hours	Oenocyte nuclei become filled with dark staining granules.
73–76 hours	Nuceli return to original condition as the oenocytes increase in size.

The central nervous system

13 hours	Neuroblasts first appear.
16 hours	Neural groove appears.
17 hours	Median neuroblasts arise along inner margin of the neural groove.
26 hours	Ventral ganglia consist of lateral cell masses separated by the neural groove.
28 hours	Neuropile begins to appear in the dorsal regions of the developing ganglia.
29 hours	Cross commissures appear in the ventral ganglia.
31 hours	Formation of the longitudinal connectives is completed.
35 hours	Fusion of the gnathal ganglia to form the subesophageal ganglion is completed. Neuropile becomes continuous between the lateral brain lobes.
37 hours	Fusion of the lateral halves of the ventral ganglia is completed.
39 hours	Fusion of the eighth, ninth and tenth abdominal ganglia is completed.

The stomatogastric system

28 hours	Frontal ganglion arises from the dorsal wall of the stomodaeum.
29 hours	Formation of the stomodaeal glands begins.
35 hours	Recurrent nerve contacts stomodaeal glands.
36 hours	Stomodaeal glands reach maximum size.

The stemmata

30 hours	Retinal cells arise from modified ectoderm cells.
33 hours	Stemmatal nerve becomes continuous with retinal cells.
46 hours	Crystalline lens forming cells arise.

The reproductive structures

8 hours	Germ cells can first be distinguished in the germ band.
9 hours	Germ cells completely invaginated.
21 hours	Germ cells completely separated into two lateral groups.
30 hours	Formation of the gonads begins as the germ cells become surrounded with mesoderm.
48 hours	Gonads reach definitive position in fifth abdominal segment.

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PREDATORS OF OLIGONYCHUS PRATENSIS (BANKS), TETRANYCHIDAE[1]

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Oligonychus pratensis (Banks) has been found increasingly more numerous and widely distributed in the Lower Rio Grande Valley of Texas since it was found on sorghum in 1953 (Griffith and Wene). Several possible predators were found closely associated with the mite during May 1955 on field corn north of Weslaco, Texas. A survey study of these predators was undertaken to determine if they fed on the mite and to determine to some degree their natural control of the mites.

The mite was described in 1912 from a few females taken on timothy grass at Pullman, Washington. Because of lack of additional information on the mite McGregor (1950) did not include it in his key. Harvey (1954) reported the mite to be the most injurious pest of wheat in eastern New Mexico during the 1952–53 season. Malcolm (1955) reported on the biology and control of the mite on grasses in Washington. Pritchard and Baker (1955) listed the mite in several states on various host plants. Walter and Wene (1956) reported the increasing importance of the mite in the Lower Rio Grande Valley area and in other states.

Oligonychus pratensis was found on the undersurface of corn, sorghum and Rhodesgrass leaves. Although the mite was difficult to find in most fields during the fall of 1955, it was easily found in at least one section of every corn and sorghum field examined during the spring season. *Tetranychus telarius* (L.) was found in very low numbers on corn and sorghum leaves in addition to the much higher numbers of *O. pratensis*.

Natural enemies of the mite have been reported by a few authors. Harvey (1954) reported the six-spotted thrips, *Scolothrips sexmaculatus* (Perg.), as a possible important predator since the mite populations on wheat in dieldrin-treated plots became significantly higher than in the check plots. Malcolm (1955) found *Hippodamia convergens* Guer. and *Coccinella transversoguttata* Fald. in substantial numbers in the early spring. He also found *Stethorus picipes* Casey and the predaceous mites, *Typhlodromus fallacis* (Garman) and *T. cucumeris* Oudms., to feed on the mite.

The most important predator during the late spring period in this area was the mite-feeding beetle, *Stethorus punctum* (Lec.). This beetle was found in large numbers in every field where the mite was present except at King Ranch, Kingsville, Texas where a *Stethorus* sp., probably *atomus* Csy. was found around a small population of *O. pratensis* on corn. *S. atomus* is the commonly-found *Stethorus* on citrus in this area that feeds on *Eutetranychus banksi* (McG.). The convergent lady beetle, *Hippodamia convergens* Guer., and *Orius insidiosus* (Say) were also found at the King Ranch location.

Six-spotted thrips were found very commonly at every location in this area. It was not observed to feed as voraciously as *S. punctum*

[1]Technical Contribution No. 2361.

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since the latter moves more continuously from mite to mite to feed and requires much less time to consume its prey. *Stethorus atomus* has been observed to move in apparently after *E. banksi* builds up to an apparently sustaining population on citrus. Indications are that this also occurs with *S. punctum*, while the six-spotted thrips is present in the lower mite population.

Other predators of the mite which were more common in the spring than in the fall period were *O. insidiosus;* the convergent lady beetle; the lacewing, *Chrysopa rufilabris* Burm.; and the ashy gray lady beetle, *Olla abdominalis* (Say). Although aphids were found in fields where these predators were observed, many were observed feeding almost exclusively on the high mite populations. *Orius insidiosus* was observed in only one field in the fall and then feeding on aphids.

Predaceous mites were also observed to feed on *O. pratensis*. *Thyphlodromus fallacis* (Garman), family Phytoseiidae, was taken in only one sample. *Pronematus ubiquitus* (McG.), family Tydeidae, was found in almost every sample. However, it was not until the fall season that this nervous, orange-colored mite was found to be predaceous on newly-hatched larvae.

The most common predatory mite was an undescribed species of Phytoseiidae. The description is as follows:

Typhlodromus mesembrinus, new species

This mite is a member of the *finlandicus* complex in which the pre-anal setae are arranged in a transverse row on the anterior margin of the anal plate. It is easily differentiated from *finlandicus* in having certain setae on legs III and IV slightly expanded and rounded distally rather than sharp. The genu of leg III has a stout blunt dorsal seta, longer than the others. The genu of leg IV has a stout, distally expanded seta dorsally and a shorter, blunt lateral seta; the tibia has a stout distally expanded seta; the tarsus has a proximal distally expanded seta; all expanded setae are stronger and longer than the normal leg setae. There is a total of seventeen pairs of dorsal setae; there are 9 pairs of marginal setae on the dorsal shield, and marginal setae 7 and 8 and dorsal median seta 2 form an equilateral triangle. The dorsal reticulate pattern is faint. The anal plate is narrower than the genital plate, and the three pairs of preanal setae lie in a transverse row anterior to the pair of well developed pores. The body of the female is 400 microns long. A strong seta, slightly expanded and rounded distally is to be found on the genu, tibia, and tarsus of leg IV, and on the genu of leg III respectively. Specimens taken from citrus have sharp setae on genu III.

Type habitat.—Associated with *Oligonychus pratensis* (Banks) on undersurface of corn and sorghum leaves.

Type locality.—Mercedes, Texas.

Type.—U. S. National Museum No. 2224.

Holotype.—A female collected from under surface of corn leaf, Mercedes, Texas, May 23, 1955 by H. A. Dean.

Paratypes.—Five females with same data as holotype.

The mites were found to feed on *O. pratensis* taken from corn on May 30, 1955 at Weslaco, Texas, and from sorghum on July 5, 1955, just west of Harlingen, Texas. The mite is named *Typhlodromus mesembrinus* as a southern form of the *finlandicus* complex.

The mites worked almost exclusively under the webs, spreading from the base of the leaves as the populations increased. Mites and their eggs under the fringes of the web were not readliy accessible to the predators. In order to reach these mites and eggs, predators would advance by pushing up the web with their back. It was very uncommon to find predators tangled in the webs.

SUMMARY

A survey study disclosed nine predators of *O. pratensis* found on corn, sorghum and Rhodesgrass during 1955. *Stethorus punctum, Scolothrips sexmaculatus, Orius insidiosus, Hippodamia convergens, Chrysopa rufilabris, Olla abdominalis, Typhlodromus fallacis, Pronematus ubiquitus* and *Typhlodromus mesembrinus* were found to feed on the mite. *S. punctum* was found to be the most important single factor feeding on higher populations of the mite while *S. sexmaculatus* was commonly found around low and high infestations of the mite.

Typhlodromus mesembrinus is described as a new species of the *finlandicus* complex.

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THE EUROPEAN CORN BORER, PYRAUSTA NUBILALIS (HUBN.), AND ITS PRINCIPAL HOST PLANT

III TOXIC FACTORS INFLUENCING LARVAL ESTABLISHMENT[1]

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That plant tissues may contain substances which are toxic to insects is well known. The insecticidal effectiveness of plant-derived materials such as rotenone, pyrethrums, nicotine, and many others, testifies to both the existence and the potency of such biological compounds. The role that toxic plant products might play in the resistance of a given plant toward infestation by insects has not been investigated to any great extent. In a few instances, however, some degree of correlation has been shown between host plant resistance and the presence of deleterious substances in the plant tissues (reviewed by Painter (1951) Kuhn and Low (1955)).

The inadequacies of young corn plants as larval hosts for the European corn borer were discussed in an earlier paper of this series (Beck, 1956b). The results of many workers indicate that newly hatched borer larvae placed in the whorls of small corn plants, under either field or laboratory conditions, undergo a high mortality, with only about 20 percent surviving the first 72 hours. Those larvae which survive show a growth rate which is lower than that of larvae feeding on the tissues of larger plants. The high initial larval mortality is not explicable on the basis of nutritional deficiencies in the host plant tissue, as extreme nutritional deficiencies did not cause comparable mortality among newly hatched larvae placed on purified diets under laboratory conditions (Beck 1950, 1956a). No evidence has been found that larval feeding is dependent upon token stimuli which might be missing from small corn plants (Beck 1956a, b). These considerations led the authors to test the hypothesis that the high mortality and low growth rate of borers feeding on the tissues of young corn plants is attributable to the presence of one or more toxic substances present in the plant tissues. It was subsequently found that such materials occur in corn plants, and this paper is a report on part of that research.

METHODS

The plant extracts used in the experiments of this study were prepared from corn plants grown in plots on University of Wisconsin farmlands. Plant height was determined by measuring the distance from the base of the plant to the tip of the longest leaf. The tassel ratio method of Luckman and Decker (1952) was not routinely employed, because many of the plant sizes used were too small to display a differentiated tassel. The plants to be utilized were harvested at the same time each day and were either frozen or processed within an hour after harvesting. To prepare the starting plant concentrate, the plant tissue was homogenized with two milliliters of water per gram of fresh tissue in a Waring Blendor. The homogenate was boiled for five minutes and filtered with washing. The fibrous residue was discarded, and the filtrate was concentrated under reduced pressure until one milliliter of concentrate represented two grams of fresh plant tissue. The pH of the concentrate was adjusted to 6.5 and any precipitate was removed by centrifugation. Further chemical treatment and fractionation of the plant concentrates are described in the section in which the results are presented.

Bioassays of plant concentrates for growth inhibitory or toxic effects were carried out. Assays with borer larvae involved incorporating the plant concentrates into a modification of the basal purified diet previously developed and using the techniques for aseptic rearing described by Beck and Stauffer (1950). The assay diet is shown in Table I. Addition of plant fractions was always in aqueous solution and at the expense of the water normally added to the diet. The sugar (glucose) and protein (casein) content of the assay diet was made to approximate that of corn leaves.

The borers were reared on the test diets for ten days, at the end of which time the larvae were weighed. In most assays the average larval weights at the end of ten days were used to estimate the activity of the plant fractions tested. In experiments in which growth rates were to be determined, the widths of the larval head capsules were measured as previously described (Beck, 1950). Feeding response experiments were run by the method described in a previous paper (Beck, 1956c).

The mold *Penicillium chrysogenum* was also used as an assay organism. In these bioassays the culturing medium used was a two percent agar containing six percent honey, one percent Bacto peptone, and the plant concentrates to be

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[3]Department of Botany.

tested. Petri dishes containing the medium were innoculated with approximately 12 penicillium spores and incubated at 30° C. for 72 hours. Colony diameters at the end of the incubation period were used as the criterion of growth.

TABLE I

COMPOSITION OF PURIFIED DIET FED EUROPEAN CORN BORER LARVAE IN ASSAYS FOR GROWTH INHIBITORS CONTAINED IN CORN PLANT TISSUES

CONSTITUENT	AMOUNT (G)	CONCENTRATION (MG/G DIET)
Water	60.0	857.1
Bacto-Agar	1.6	22.8
Fibrous cellulose	0.8	11.4
Corn leaf factor concentrate	2.0	28.6
Glucose	0.5	7.1
Casein	2.5	35.7
Yeast powder	2.0	28.6
Choline	0.03	0.4
Corn oil	0.1	1.4
Cholesterol	0.2	2.8
Wesson salts	0.3	4.3
Total	70.03	1000.2

The *Penicillium* bioassay was found to have some advantages over the borer assay; it was simpler, faster, and yielded more consistent results. The use of duplicate assays employing organisms so different in their biological properties had the disadvantage of necessitating the assumption that the two organisms were reacting to the same compounds in the material being assayed. The validity of this assumption has been borne out by the results thus far obtained.

RESULTS

Plant concentrates were prepared, as described above, from the whorl leaves of plants showing an extended height of 27 inches. The "borer resistant" inbred Wis. 22RB and the "borer susceptible" inbred Wis. 204 were used. The concentrates were added to borer diets in amounts such that one gram of medium was equivalent to either one or two grams of fresh leaf tissue. The borers were maintained for ten days, at the end of which time they were weighed and the experiment was terminated. Results which are typical of this type of experiment are shown in Table II.

These data show that the addition of aqueous concentrates of the whorl leaves of small corn plants to purified diets was deleterious to borer growth. The growth inhibition caused by concentrates of the "resistant" inbred was greater than that brought about by those of the "susceptible" inbred. The inhibitory activity present in the plant concentrates was termed the *resistance factor(s)*, and intensive research was begun on its chemical characteristics, biological properties, and its role, if any, in the relationships between the larvae of the European corn borer and the corn plant.

The activity of the resistance factors against newly hatched borer larvae is shown in figure 1. With increasing dietary levels of a resistance factor concentrate prepared from 25-inch corn plants, larval mortality rose sharply and larval growth was strongly inhibited. Resistance factor activity was found to vary among the plant concentrates prepared, depending on the plant tissues used as well as on the age and variety of the corn plants.

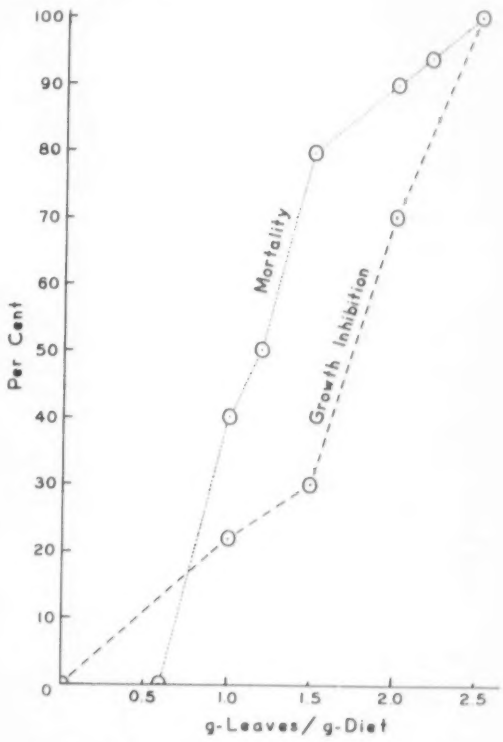

FIG. 1. Effect of aqueous concentrates of young corn leaves on the growth and survival of European corn borer larvae. (10 day assay).

The biological activity of the resistance factor has been found not to be confined to that of an inhibitor of European corn borer growth and survival. It acted as a growth inhibitor against all organisms thus far tested. Resistance factor preparations added to the culturing media have been found to inhibit the growth of the following organisms in addition to the European corn borer: the German cockroach, *Blattella germanica* (L.), the southern armyworm *Prodenia eridania* (Cram.), the fungi *Rhizopus stolonifera*, *Penicillium chrysogenum*, and *Diplodia zeae*, the

bacterium *Staphylococcus aureus*, and cucumber stem tissue cultured by the method of Casida and Allen (1951). It was after testing the above organisms that the *Penicillium* bioassay for resistance factor was developed. Since the corn leaf concentrates tested against these several organisms were exceedingly crude from a chemical standpoint, there was no assurance that the different organisms were inhibited by the same components of the concentrate. Subsequent experimentation has shown that some of the toxins present are active against both the borer larvae and *Penicillium*.

TABLE II

GROWTH OF EUROPEAN CORN BORER LARVAE MAINTAINED FOR TEN DAYS ON PURIFIED DIETS CONTAINING AQUEOUS CONCENTRATES OF CORN WHORL LEAVES

	Control	Corn Variety and Concentration (g leaves/g diet)			
		Wis. 22Rb (resistant)		Wis. 204 (susceptible)	
		1 0	2 0	1 0	2 0
Average larval weight (mg)	22 0	16 5	4 5	23 2	9 2
Growth inhibition (%)	0	25	79 5	0	53 7

TABLE III

INFLUENCE OF RESISTANCE FACTOR PREPARATIONS ON THE FEEDING BEHAVIOR OF NEWLY HATCHED EUROPEAN CORN BORER LARVAE

Relative Resistance Factor Activity of Plant Preparation*	Larval Distribution After 24 Hours	
	Control Diet (%)	Treated Diet (%)
none	35	65
low	27	73
high	29	71
very high	40	60

*Resistance factor activity determined by *Penicillium* assay.

The finding that resistance factor concentrates inhibited the growth of a variety of organisms was taken as evidence that the effect on corn borer larvae was physiological and not behavioristic. This point was tested, however, by a study of the effect of resistance factor preparations on the feeding behavior of newly hatched borer larvae. The data of Table III were obtained from experiments in which newly hatched borer larvae were given a choice of two diets on which to feed. The distribution of larvae between the diets at the end of 24 hours was taken as a measurement of the relative influence of the experimental variables on the maintenance of feeding (Beck, 1956c). In the present experiments, the results indicated that larval feeding was enhanced by the plant extracts tested without regard to their resistance factor activities. The plant extracts used were prepared from whorl leaves and represented different stages in the chemical purification of the resistance factor.

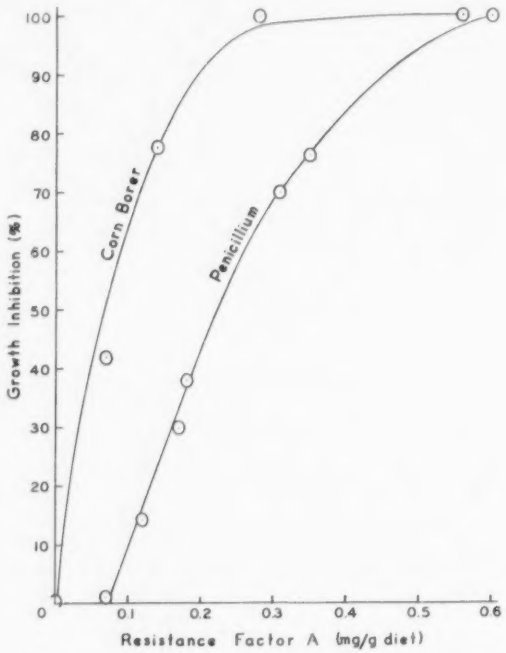

FIG. 2. Inhibition of the growth of *Penicillium chrysogenum* and European corn borer by crystalline Resistance Factor A.

Chemical fractionation procedures have been used in an effort to isolate and identify the inhibitors contained in the aqueous corn leaf concentrates. One of the first steps tried in the purification study was an extraction with an organic solvent. Using a liquid-liquid continuous extracter, samples of the aqueous concentrates were extracted with ether or benzene for from 48 to 72 hours. The fractions so obtained were assayed for resistance factor activity against both the borer and *Penicillium*. Results of the borer assay are presented in Table IV. The *Penicillium* assays yielded comparable results. As the solvent extraction proceeded, a precipitate formed in the aqueous fraction. This material was removed by centrifugation and assayed; as shown in Table IV, it had a growth stimulating effect on borer larvae. Its effect on *Penicillium* was a very slight growth stimulation. The assay

data show the presence of at least two inhibitors, one or more being ether and benzene soluble and the other(s) being water soluble but insoluble in the organic solvents. For the sake of convenience, the ether- and benzene-soluble factor was called Resistance Factor A, and the solvent-insoluble factor Resistance Factor B. The crude concentrates appeared to have approximately equal A and B activity.

Further fractionation and purification studies have been made on Resistance Factor A, and it has been isolated in pure crystalline form. At the date of this writing, its chemical and physical characterization is not yet complete. Information on the chemical structure and properties of Resistance Factor A will be published at a later date. The biological activity of crystalline Resistance Factor A against borer and *Penicillium* growth is shown in figure 2. The data of this figure show that this substance is a powerful inhibitor of growth and that its effects on the growth of the two assay organisms is essentially similar.

Resistance Factor B has not yet been isolated in pure form; neither has it been established that Factor B is a single substance. Some fractionation and assay data obtained have been indicative of there being two active inhibitors in the solvent-insoluble fraction B. Because Resistance Factor A has proved to be more easily separated from the aqueous concentrate than has Factor B, its importance in the relationship between the borer and the corn plant has been emphasized in the research thus far. In most of the results presented below, only Factor A is considered.

If the so-called resistance factors have, in fact, any appreciable influence in determining the adequacy of corn plants as borer hosts, a demonstrable correlation must exist between the resistance factor activity of the tissues of corn plants of different ages and varieties and the performance of borer larvae on those plants under field conditions. Toward this end, a series of corn plants of the "borer resistant" dent corn inbred W210D were assayed for Resistance Factor A activity. The plants used were all planted on the same date, but were harvested and frozen at different times to provide a series of plants of different extended heights. Although the entire aerial portions of the plants were processed to yield a Resistance Factor A concentrate, the amounts of the concentrates used was such that the amount of plant tissue represented in each assay was uniform (10g dry weight). The tissue titer of Factor A activity declined as the extended height of the plants increased (figure 3). This

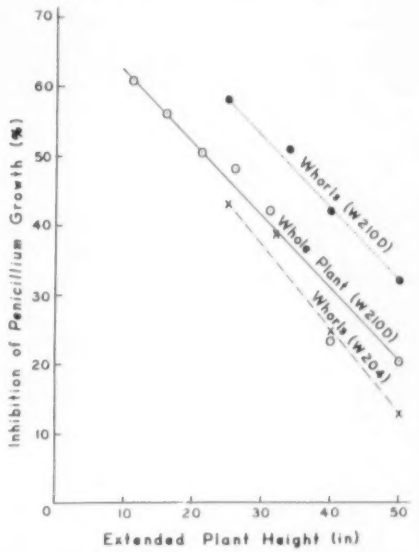

FIG. 3. Resistance Factor A activity of tissues of two dent corn inbreds.

figure also shows the results of a similarly designed series of assays in which only the whorl leaves and developing tassels were used. In these assays two inbreds were used, the "borer resistant" W210D and the "borer susceptible" W204. A declining concentration with plant size was clearly demonstrated. A varietal difference was also indicated, in that the Resistance Factor A level was higher in the whorl tissues of W210D than W204. The Resistance Factor A concentration of the whorls of W210D was somewhat higher than that of the plant as a whole.

These findings are fully consistent with the

TABLE IV

INHIBITION OF LARVAL GROWTH OF EUROPEAN CORN BORERS MAINTAINED FOR TEN DAYS ON PURIFIED DIETS CONTAINING DIFFERENT FRACTIONS OF AQUEOUS CONCENTRATES OF WHORL LEAVES OF YOUNG CORN PLANTS

PREPARATION	INHIBITION BY CRUDE CONCENTRATES	FRACTIONATION SOLVENT	SOLVENT SOLUBLE	GROWTH INHIBITION OF FRACTIONS		TOTAL RECOVERED INHIBITION
				SOLVENT INSOLUBLE PPT	SOLVENT INSOLUBLE SUPERNATANT	
	(%)		(%)	(%)	(%)	(%)
1	45 3	ether	26 2	−20 1	38 5	44 6
2	47 9	benzene	25 8	−10 2	27 3	42 9

hypothesis that the biological activity of Resistance Factor A is responsible for at least part of the varietal resistance of the corn plant to the establishment and survival of the European corn borer. The data also tend to support the postulate that varietal resistance is in part dependent on the growth stage at which infestation occurs. A number of workers have pointed out that borer-resistance in corn plants is a transient phenomenon very closely associated with plant growth stages, and that varietal differences in borer resistance may be explicable in terms of differences in developmental rates, (Huber 1939, Beard 1943, Beck 1951 and others). This is borne out by the data presented in figure 3; *e.g.*, the Resistance Factor A activity in the whorl tissue of 40-inch W210D Plants was about the same as in the whorls of 26-inch plants of the more susceptible W204. Under the conditions prevalent during this work, the growth characteristics of the two inbreds were quite similar; at a given extended height, essentially identical tassel ratios were observed. However, if varietal differences in resistance were only the result of differential developmental rates, the regressions of Resistance Factor A activity on plant height should show converging curves for the whorl tissue of the two inbreds tested. As shown in figure 3, however, the curves obtained were essentially parallel. Such data indicate that the magnitude of the varietal difference was constant over the range of plant sizes tested. The data obtained on this point are limited, and the role of Resistant Factor(s) B is yet to be defined; so no generalization is possible other than that the activity of Resistance Factor A tends to decline as the plants grow larger and that varietal differences were found.

The data discussed above were obtained from corn planted in mid-May, which is the time of normal agricultural planting in southern Wisconsin. Other plots were planted in late May and mid June, and Resistance Factor A activity of the tissues of these late planted corn plants showed an irregular pattern not always consistent with that obtained from the early planted corn. These results were interpreted as indicating possible importance of environmental factors on the manifestation of resistance. This point is being studied currently, as it is thought that it may be of great importance to an understanding of the instability of borer resistance observed by Turner & Beard 1950, Turner 1951, and others.

A number of inbred lines and single crosses of dent corn have been tested qualitatively for the presence of Resistance factor in the whorl tissue. Without exception, good resistance factor activity was observed. A systematic quantitative survey of various lines must, if it is to be meaningful to the resistance problem, await clarification of the role of Factor(s) B and environmental influences.

SUMMARY

1. In a study of the factors influencing the establishment and survival of European corn borer larvae on corn plants, it was found that two or more toxic substances occur in the plant tissues. These toxicants have a deleterious effect on the growth and survival of borer larvae, and also have an inhibitory effect on the growth of a number of other organisms.

2. One of the toxic substances has been isolated in pure form and is called Resistance Factor A. The Resistance Factor A content of two dent corn inbreds was found to differ in agreement with their relative resistance to infestation by the borer under field conditions. Resistance Factor A content of whorls and whole plants was found to decline with increasing plant height, as does resistance to borer establishment.

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NOTES ON TYPES OF MOSQUITOES IN THE HUNGARIAN NATIONAL MUSEUM (DIPTERA, CULICIDAE)

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Through the kind cooperation of Dr. F. Mihályi, of the Hungarian National Museum, I have been able to examine types of a number of heretofore unrecognized or dubiously identified mosquitoes described by F. V. Theobald (1905). In addition, types of three species described by F. W. Edwards (1922) were sent me by Dr. Mihályi. This paper presents notes on the 28 nominal species and one variety described in the publications cited. Type material of two of the Theobald species was not available for study and is apparently lost.

In the notes that follow, the page reference to the appropriate publication is given following each species name. Unless otherwise stated the specimen examined agrees, in my opinion, with the currently accepted interpretation of the species. For each species discussed I give a reference to an adequate recent description. New synonymy is summarized near the end of this paper.

Kerteszia boliviensis Theobald, p. 66.

Female holotype. The hind legs are missing, the dark scales on the dorsum of the abdomen are not particularly concentrated on the hind margins of the segments, and the white scales of the sternites are nearly all gone. (Lane, 1953, p. 279, as *Anopheles*.)

Bironella gracilis Theobald, p. 69.

Male, labeled type. This species was described from three males. The specimen received is rather moldy, but otherwise in good condition.

There has been considerable confusion concerning the identity of this species, as well as the name to be used for it. To take up the nomenclatorial matter first, Christophers (1924, p. 16) placed *Bironella gracilis* Theobald in the genus *Anopheles* and in doing so noted that it was preoccupied in *Anopheles* by *A. gracilis* Doenitz 1902. He therefore proposed the name *Anopheles bironelli* to replace *A. gracilis* (Theobald). This name was accepted by a number of workers who used the generic name *Anopheles*, but those who retained *Bironella* continued to use *gracilis*. According to the Bulletin of Zoological Nomenclature 4: 121, 1950, a rejected homonym, even though a secondary one, cannot be resurrected; therefore *Bironella gracilis* Theobald falls as a synonym of *Bironella bironelli* (Christophers).

Swellengrebel and Rodenwaldt (1932) figured the terminalia of the species under the name *Anopheles bironelli* (fig. 15, and plate 2, fig. 5), but Bonne-Wepster (1951, p. 71) assigned the name *gracilis* to a species having different terminalia and proposed the name *Bironella occulta* for the species having terminalia like those drawn by Swellengrebel and Rodenwaldt. I cleared the terminalia of this syntype male of *Bironella gracilis* and find that they agree closely with the Swellengrebel and Rodenwaldt figures, but differ considerably from those of Bonne-Wepster (1953, p. 53). The shape of the dististyle and apex of the basal arms agree very well. The type does not show a basal lobe on the basistyle as figured by Swellengrebel and Rodewaldt, but there is a very pronounced, sharply demarcated, triangular patch of setae in this position, and I believe that this was drawn to look like a detached basal lobe.

In the U. S. National Museum are a number of specimens that agree well with this syntype male of *gracilis*. I have seen none that appear more like the drawings of Bonne-Wepster (1953) or Lee and Woodhill (1944, p. 55) than they do like the type or like Swellengrebel and Rodenwaldt's drawings. It seems quite possible that only one species is involved and that the differences are due to the inaccuracies of drawings rather than to actual differences in the specimens.

Stegomyia W-alba Theobald, p. 74.

Female holotype. This is in good condition except for loss of one leg of each pair and tarsomeres from all but the fore leg. (Barraud, 1934, p. 232, as *Aedes*.)

Stegomyia pseudonivea Theobald, p. 75.

Female holotype. This is in good condition with all but one leg intact. It agrees well with the original description of *Aedes subniveus* Edwards 1922, which has been synonymized under it.

Skusea culiciformis Theobald, p. 77.

The type of this species is lost, according to Dr. Mihályi, only the labeled pin being left. This has been considered by Edwards (1924, p. 386) as probably belonging to *Aedes*, subgenus *Pseudoskusea*, and he identified a specimen from Moa I., N. Australia, as Theobald's species.

Skusea funerea var. *ornata* Theobald, p. 79.

Female holotype. The specimen is in good condition except that all tarsi are incomplete

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except the right hind one. (King and Hoogstraal. 1949, p. 118, as *Aedes lineatus* (Taylor.)

Lepidotomyia albosculellata Theobald, p. 80.

Female, labeled type, Simbang, Huon Golf, New Guinea. This species was described from two females, the second being from Friedrich-Wilhelmshafen. The specimen received is in good condition, with all but one leg intact. (Knight and Hull, 1953, p. 457, as *Aedes*.)

Culex biroi Theobald, p. 82.

Female labeled holotype, male labeled allotype. This species was described from three females and three males from Bombay. The male received lacks the head. No holotype was originally designated, and no lectotype has been published. It seems advisable to withhold selection of a lectotype until the entire series can be studied and the best specimen, the one in closest agreement with the original description or the one least upsetting to current nomenclature, can be selected. This is particularly important since the identity of *Culex tritaeniorhynchus* Giles, with which this species has been synonymized, is somewhat uncertain.

Culex trimaculatus Theobald, p. 86.

Female holotype. This is in good condition except for the loss of one hind leg and part of the tarsus of one fore leg. (Barraud, 1934, p. 264, as *Aedes*.)

Culex pullus Theobald, p. 87.

Female holotype. This is in good condition except that the pin goes through both pleura and most of the distal tarsomeres are missing. The dark markings of the pleura can be seen, however. (King and Hoogstraal, 1946, p. 149.)

Culex quasimodestus Theobald, p. 88.

Female holotype. The head and one fore leg are missing from this specimen, but it is otherwise in good condition. The venter is entirely pale scaled, and I believe it to be *Culex molestus* Forskal. rather than *C. pipiens* Linnaeus

Lophoceratomyia fraudatrix Theobald, p. 94.

Male labeled holotype and female labeled allotype, Friedrich-Wilhelmshafen. This species was described from a large series of males and several females from Friedrich-Wilhelmshafen and Stephansort, Astrolabe Bay, New Guinea. The labels on these two specimens, written by Theobald, are both *Lophoceraomyia fraudator*, this spelling agreeing with that of the generic name as originally published, and the spelling of the specific name being as used by Edwards (1922, p. 281).

The male has the antennal tufts typical of the *fraudatrix* group. The large tuft on the sixth flagellar segment is bicolored. The terminalia, which I cleared, do not seem to agree well with any published figures although they are close to those drawn by Baisas (1935, p. 172) for *josephineae*. Many of the specimens determined as *fraudatrix* in the U. S. National Museum have the terminalia quite different from those of the holotype, but one from New Guinea agrees very well. Until the entire group can be carefully revised so that the extent of the variation in the terminalia can be determined, little more can be said about this species, nor do I consider the selection of a lectotype desirable.

Lophoceratomyia brevipalpus Theobald, p. 96.

Male holotype. This specimen is in good condition except for lacking some tarsomeres. Theobald's label, in this case also, has the generic name spelled without the *t*. The terminalia of this type agree very well with those described by Edwards (1928, p. 278) for *Culex eminentia* (Leicester). The palpi are scarcely longer than half the length of the proboscis, and the small tuft on flagellar segment 6 is not yellow. Edwards treated this as a synonym of *eminentia* (Leicester) 1908, thinking that it was preoccupied by *Culex brevipalpis* (Giles) 1902, but since under the Rules a nominative ending and an adjectival ending are not homonymous, we must resurrect *Culex brevipalpus* (Theobald) and place *C. eminentia* (Leicester) as a synonym.

Trichopronomyia annulata Theobald, p. 98.

Male holotype. The specimen is in fair condition. The palpi are missing and all but one hind tarsus is incomplete. (Edwards 1924, p. 393, as *Culex squamosus* (Taylor).)

Melanoconion ornatus Theobald, p. 100.

Female holotype. This specimen is in fair condition. Only three legs are present and only the fore one is complete. The proboscis is broken and most of the antennal segments are gone. (King and Hoogstraal 1955, p. 7, as *Culex*.)

Melanoconion pallidiceps Theobald, p. 101.

Male holotype. This is in good condition and I have cleared the terminalia. It agrees very well with *Culex perkinsi* Stone and Penn (1948) from the Solomon Islands; so the name of the species should be *Culex* (*Acallyntrum*) *pallidiceps* (Theobald). The type locality is Friedrich-Wilhemshafen, New Guinea.

Grabhamia longisquamosa Theobald, p. 103.

Female holotype. This specimen is in fairly good condition. Edwards (1932, p. 141) was probably correct in treating this as a variety of *caspius*. The posterior half of the scutum is considerably denuded but the scales of the anterior half are present and show no trace of the white lines found in *Aedes caspius*. The pale

scales of the wing are rather evenly scattered, in this way differing from the related *Aedes dorsalis*. The abdomen has the pale scales scattered, with the mid-dorsal stripe not very evident and the lateral spots not distinctly white. The claws are as in *caspius*, not as in the related *A. mariae*.

Grabhamia maculosa Theobald, p. 105.

Female, labeled *Culex maculosus* Type. There is no doubt that this is one of the five females described by Theobald under the name *Grabhamia maculosa*. This proves to be a synonym of *Aedes detritus* (Haliday). The name *maculosa* was omitted from Edwards' (1932) catalogue.

Mansonia septempunctata Theobald, p. 107.

Female holotype. This is in excellent condition (Edwards 1930, p. 542).

Leptosomatomyia litoralis Theobald, p. 110.

Male holotype. The specimen is rather moldy, one wing and three legs are missing, and the sternites are almost completely concealed by the folding over of the tergites so that it is difficult to see that the abdomen is not dark banded ventrally. The terminalia are in balsam on a strip of celluloid pinned below the specimen. (Edwards 1922, p. 94, as *Aedes aurimargo* Edwards.)

Uranotaenia testacea Theobald, p. 113.

Female labeled type. This species was described from two females. The specimen received is in fair condition except that only the hind legs are complete and one wing is missing. (Barraud 1934, p. 74.)

Uranotaenia atra Theobald, p. 114.

Female holotype. This bears the original data, "N. Guinea, Muina, Biró 1900." It proves to be identical with *Uranotaenia nigerrima* Taylor (1914, p. 203), one of the most abundant species of New Guinea. Theobald failed to note the entirely dark postpronotum and the large dark spot in front of the wing base on the scutum, characteristic of this species, but the rest of the description fits well. Edwards (1913, p. 238) published on the type of *atra*, stating that it was moldy and rubbed and scarcely recognizable, but he considered *U. ceylonica* Theobald 1910 to be the same species. The specimen is somewhat moldy, but it is scarcely rubbed and it is surprising that Edwards also failed to note the black spots on the thorax. The absence of the broad bright blue scales on the thorax of *atra* easily distinguishes it from *ceylonica* (= *atra* of authors).

Since *Uranotaenia atra* has been consistently applied to a different species than its type, as pointed out above, it becomes necessary to resurrect a name to replace *atra* of authors, not Theobald. The oldest available name is *Uranotaenia coeruleocephala* var. *lateralis* Ludlow (1905, p. 385). The four syntype females of this name are in the U. S. National Museum, and I select as lectotype of *lateralis* the specimen labeled by Ludlow as type from Cottabato, Mindanao, P. I. June. There seems to be no reason to doubt the above synonymy. The description by Belkin (1953, p. 324) of the species (as *atra*) from the Solomon Islands is an excellent one.

Phoniomyia bimaculipes Theobald, p. 114.

This was described from three females. Dr. Mihályi found no type in the collection.

A specimen in the British Museum marked as cotype bears the data, "Moroka 1300 m. Loria VII–XI 1893" and the label, "Mus. Civ. Genova." Because of this last label it may not have been one of the original specimens from the Hungarian Museum. This specimen differs from the description given by Lee (1946, p. 231) in having the clypeus scarcely dark brown, the tori distinctly orange brown, two spiracular setae, the mesepimeron practically denuded, the mid and hind femora without a silvery line anteriorly, and the spots on the hind femora only slightly larger than those on the fore and mid femora.

Phoniomyia indica Theobald, p. 115.

Male holotype. Most of the abdomen is gone from the specimen. Some of the important characters are: last two segments of antenna elongate; palpi scarcely longer than clypeus; proboscis 1.3 times as long as fore femur; vein R_{2+3} 0.6 as long as vein R_2; mid femora dark beneath with only a short, narrow line of pale scales from base on posterior surface; white of hind femora extending about two-thirds from base.

A comparison of this with the type of *Heizmannia scintillans* Ludlow (1905, p. 130) shows no significant differences, and I think they represent one species. Since *scintillans* was published in April and *indica* in June the latter falls as a synonym.

Phoniomyia magna Theobald, p. 117.

Female holotype. The specimen is somewhat rubbed but is otherwise in good condition. (Lane 1953, p. 836.)

Polylepidomyia argenteiventris Theobald, p. 118.

Female, labeled type. This species was described from five females. The specimen received bears the labels, "Type / New Guinea Paumomu R. Loria IX–XII 1892 / typus *Polylepidomyia argenteiventris* Theob. / *Polylepidomyia argenteiventris* F. V. Theobald Type / Coll Mus Nat. Hung." Since Theobald mentions a considerable variation in size and apparently the relative length of the palpi and proboscis, etc., it becomes desirable to select a lectotype for the species, and since this is probably the only specimen Theobald labeled as type I here designate it as lectotype.

This specimen has the palpus exactly one-sixth the length of the proboscis and extending more than twice the length of the clypeus beyond the clypeus, so that it agrees with *Tripteroides atra* (Taylor 1914, p. 190) in the key of Lee (1946, p. 228). I have also compared this lectotype with a specimen determined by Lee as *atra* and found close agreement. I think *Tripteroides atra* (Taylor) can be placed as a synonym of *Tripteroides argenteiventris* (Theobald).

Psorophora purpurascens Edwards, p. 77.

Female holotype. The specimen is in rather good condition. (Lane, 1953, p. 747, as *P. cyanescens* (Coq.).)

Psorophora varinervis Edwards, p. 78.

Female holotype. The specimen is in fair condition. (Lane 1953, p. 764.)

Armigeres lacuum Edwards, p. 97.

Male holotype. This specimen is somewhat moldy but is otherwise in good condition. The terminalia are mounted on a celluloid slip pinned below the specimen. (Lee 1944, p. 219.)

SUMMARY

Notes are presented on 25 species and 1 variety described by Theobald and 3 species described by Edwards from the Hungarian National Museum, loaned to me by Dr. F. Mihályi of that Museum. As a result of my study of this material I have noted the following new syonymies:

Bironella bironelli (Christophers) (= *B. occulta* Bonne-Wepster).
Culex molestus Forskal (= ? *C. quasimodestus* Theobald).
Culex brevipalpus (Theobald) (= *Lophoceratomyia eminentia* Leicester).
Culex (Acallyntrum) pallidiceps (Theobald) (= *Culex perkinsi* Stone and Penn).
Aedes detritus (Haliday) (= *Grabhamia maculosa* Theobald).
Uranotaenia atra Theobald (= *U. nigerrima* Taylor).
Uranotaenia lateralis Ludlow (= *U. atra* of authors).
Heizmannia scintillans Ludlow (= *Phoniomyia indica* Theobald).
Tripteroides argenteiventris (Theobald) (= *Stegomyia atra* Taylor).

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ERRATUM

On page 376 of Volume 49, July, 1956, line 32 of the left column should read as follows: and one apical hook, 2–6 in a transverse row

VARIATIONS IN THE LARVAE OF CULEX STIGMATOSOMA DYAR WITH NOTES ON SIMILAR SPECIES[1] (DIPTERA: CULICIDAE)

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INTRODUCTION

Culex stigmatosoma was described by Dyar (1907) from a large number of specimens that had been collected mostly from California. A rather general description was given for the larva, but Dyar stated that the larva would fall into the table of Dyar and Knab (1906) with *pipiens* and *cubensis*.

Howard, Dyar and Knab (1915) gave a more detailed description of the larva; they stated that five pairs of hair tufts occurred on the siphon, and that the basal tufts were included within the pecten. Dyar (1928) published essentially the same description of the larva as in the above monograph, and he apparently used the same illustration. At this time Dyar also considered *Culex thriambus* Dyar to be a subspecies of *C. stigmatosoma*. Some confusion resulted until *C. thriambus* was restored to full specific rank, but it is not believed that there is any connection between this confusion and the discrepancies in descriptions of the larvae of *C. stigmatosoma* to be cited shortly. The larvae of *C. thriambus* and *stigmatosoma* are radically different, and the descriptions that have been selected cannot be logically applied to *C. thriambus*.

If one checks several publications in which the larvae of *C. stigmatosoma* are described, it will be noted that the descriptions are quite different relative to certain features. Characteristics in which there has been most inconsistency include the number of pairs of hair tufts on the siphon, the relation between the basal tufts and the pecten, and the alignment of the tufts with respect to each other. The following descriptions and illustrations have been selected from five relatively recent publications as examples of the various ways these features have been described.

Number of Siphonal Tufts: Four pairs of hairs; five pairs of hairs; four to six pairs—usually six; five pairs of hairs; five pairs of tufts.

Relation of Tufts to Pecten: Basal tuft within pecten; pecten followed by tufts; siphonal hairs inserted beyond pecten (illustration shows basal tuft within pecten); tufts beyond pecten; basal tuft within pecten.

Alignment of Tufts: Subapical tuft out of line; subapical tuft sometimes out of line (in key, tufts must be all in line or nearly so for correct identification); subapical tuft out of line; subapical tuft slightly out of line; subapical tuft out of line.

[1]This investigation was supported by a research grant E-567 (C2) from the Microbiological Institute of the National Institutes of Health, Public Health Service. Drawings by Grace Hewitt.

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THE PRESENT STUDY

The inconsistencies in the literature relative to the larvae of *C. stigmatosoma* first came to the writer's attention when he collected a large number of larvae from two localities in west Texas. The collecting sites were two miles north and twelve miles north of Fort Davis, Jeff Davis County, Texas. Both collections were made in stagnant water in the bed of a stream known as Limpie Canyon Creek.

Some of the collected larvae were isolated from associated species and reared to adults; others were preserved for study. Some of the preserved larvae were later mounted on permanent slides, and an examination of these specimens revealed so much variation in certain features that published descriptions were checked. The remainder of the preserved specimens were then studied in more detail.

The present report is based upon a study of 87 preserved larvae and larval skins, as well as an examination of a large number of permanent slides. There is often considerable variation in the siphonal tufts and their insertions on the two sides of the siphon. And, on permanent slides in which a lateral mount is made, only one side of the siphon is clearly visible. For these reasons, specimens on permanent slides are not included in the figures given from time to time relative to the occurrence of certain features.

The most noticeable of the variations in the larvae that were studied were the same features in which there is most inconsistency in the literature, namely, the number of hair tufts on the siphon, the relationship between the basal tufts and the pecten and the alignment of these tufts with respect to each other. Various combinations of these features are shown in figs. 1 and 2.

The number of tufts varied from four tufts on both sides to six on both sides. Fifty-six of the 87 preserved larvae and larval skins had five tufts on both sides; seven had six tufts on both sides and six had four. The remaining eighteen had five tufts on one side and either four or six on the other.

Several combinations occurred with respect to the basal tufts and the pecten. The basal

tufts on both sides were within the pecten in all seven of the specimens with six tufts on both sides; the tufts were beyond the pecten in the six larvae with four tufts on both sides. Of the fifty-six larvae with five tufts on both sides,

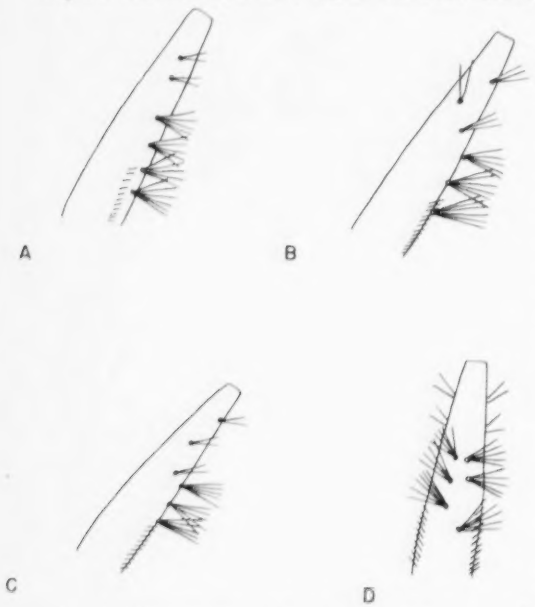

FIG. 1. Variations in siphonal tufts of the larvae of *Culex stigmatosoma* Dyar. A, B and C, lateral view of siphon; D, ventral view.

sixteen had the tufts within the pecten on both sides; in eighteen the tufts were inserted beyond the pecten. In the remaining twenty-two larvae, the basal tufts were within the pecten on one side and beyond on the other (fig. 1, D). The same type of variations occurred in the eighteen larvae with a different number of tufts on opposite sides of the siphon.

In all except two larvae, one or two of the distal tufts were definitely out of line on both sides of the siphon (fig. 1, B, C; fig. 2, E, F, G, H). In these two specimens the tufts were more or less in line on one side (fig. 1,A) although the attachments of the tufts became more lateral as one passed from the base of the siphon to the distal end. In both these cases, the subapical tuft was definitely out of line on the opposite side of the siphon.

Discussion: Most of the living larvae had pupated or emerged as adults before the writer became aware of the larval variations; consequently there was no opportunity to isolate various types of larvae to determine if correlated variations occur in the adults. However, more than fifty adults were reared from these collections, and no striking variations have been observed in these specimens.

The great degree of variation in the larvae of *C. stigmatosoma* obviously presents problems in their identification. According to some publications, the larvae of *C. stigmatosoma* and *C. tarsalis* Coq. are quite similar in some areas. In Texas, however, all specimens of these two species examined have been separable on the basis of the alignment of the siphonal tufts. The only possible difficulty the writer can conceive of at present would be an occasional permanently mounted specimen of *C. stigmatosoma* with the hairs aligned on the visible side of the siphon as in figure 1,A. As indicated previously, in all such cases examined by the writer, the subapical tuft was definitely out of line on the opposite side, but this situation might not be evident on permanently mounted larvae. Dr. John M. White, a former graduate student of the writer, recently studied the siphonal hair alignment in more than 500 larvae of *C. tarsalis* collected from many localities in Texas (MS unpublished). He found that the subapical tuft was slightly out of line in a few specimens. In no case, however, did he find this tuft inserted as far laterad as in larvae of *C. stigmatosoma*. It is thus not believed that this slight variation will cause difficulty in distinguishing these two species of larvae in this region.

In the area under consideration, larvae of *Culex quinquefasciatus* Say are the ones most likely to be confused with those of *C. stigmatosoma*. In current literature, features that have been used to distinguish the larvae of these two species include the number of siphonal tufts and their relation to the pecten, the arrangement of the tufts with respect to each other, the siphonal

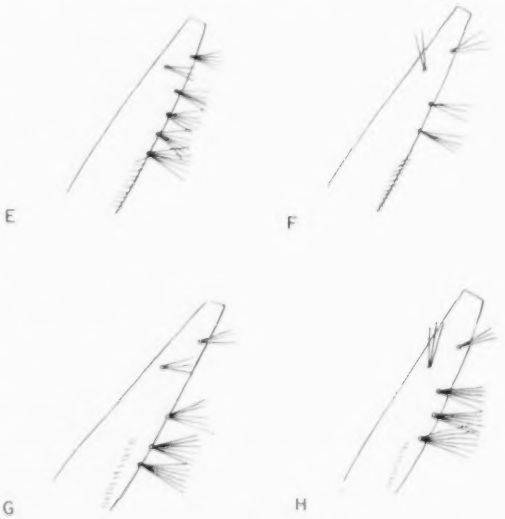

FIG. 2. Variations in siphonal tufts of the larvae of *Culex stigmatosoma* Dyar. All figures, lateral view of siphon.

index, the number of branches in the lateral hairs of abdominal segments III and IV and the relative length of the microsetae near the apex of the sclerotic plate or saddle.

The larvae of the two species in question have been described as having the following characteristics with respect to these features: *Culex quinquefasciatus:* four pairs of siphonal tufts, the subapical pair out of line; siphonal index 3: 1 to 4: 1; lateral hairs of abdominal segments III and IV usually two-branched; microsetae near apex of saddle about the same length as near the middle. *Culex stigmatosoma:* The situation with respect to the hair tufts and their relationship to the pecten has already been discussed. Published descriptions relative to other features mentioned include: siphonal index 4: 1 to 6: 1; lateral hairs of abdominal segments III and IV usually three-branched; microsetae toward dorsal apex of saddle much longer than those at the middle.

The writer has examined larvae of *C. quinquefasciatus* from several localities in Texas. In comparing these larvae with those of *C. stigmatosoma*, some overlapping of all the features listed above was found. For example, most larvae of *C. quinquefasciatus* have four pairs of siphonal tufts beyond the pecten; not infrequently, however, specimens have five pairs of tufts, and the basal tufts are sometimes included within the pecten. Also, the microsetae near the apex of the saddle in this species are sometimes noticeably longer than those at the middle.

Of the features mentioned, the most reliable is the number of branches in the lateral hairs of the third and fourth abdominal segment. In a majority of larvae examined, at least some of these hairs were two-branched in *C. quinquefasciatus*, and three-branched in *C. stigmatosoma*. There is an occasional overlap in this feature, however, and on permanently mounted larvae, some or all of the lateral abdominal hairs may be broken off or difficult to see. For these reasons, an attempt was made to discover supplementary, easily seen key features by which the larvae of the two species can be distinguished on permanent slides, as well as, fresh and preserved specimens.

Unfortunately, no easily seen single feature for differentiation was discovered. However, two characteristics were found which are different in a majority of cases, and which in combination with the branches of the lateral abdominal hairs, can be used to differentiate all the larvae examined by the writer. These features are the stirrup-shaped piece and the subdorsal (hair number 1 of Belkin, 1950, 1952) hairs of abdominal segments III and IV.

Figures 3, A through D illustrates these features in representative larvae of both species. The following couplet is proposed as a means of distinguishing between the larvae of *C. quinquefasciatus* and *C. stigmatosoma* in this area:

At least two of the following three features present: stirrup-shaped piece toothed; number 1 hairs (subdorsal) on abdominal segments III and IV with 3 or more branches; lateral abdominal hairs on segments III and IV 3-branched. . . . . *C. stigmatosoma*

At least two of the following three features present: stirrup-shaped piece untoothed; number 1 hairs (subdorsal) on abdominal segments III and IV with 1 or 2 branches; lateral abdominal hairs on segments III and IV 2-branched. . . . . *C. quinquefasciatus*

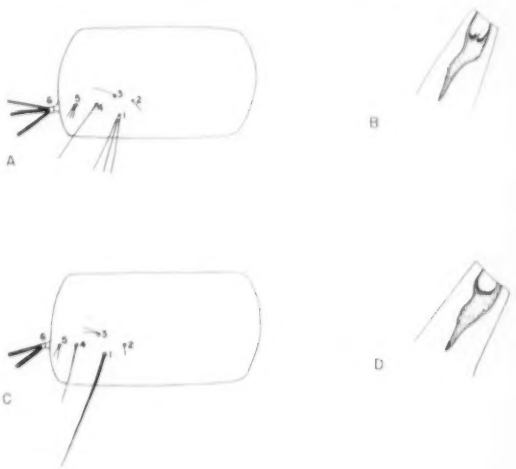

FIG. 3. Distinguishing features of the larvae of *Culex stigmatosoma* Dyar and *Culex quinquefasciatus* Say. A, dorsal view of fourth abdominal segment, and certain hairs on one side, of *C. stigmatosoma*. C, same of *C. quinquefasciatus*. B, tip of siphon and stirrup-shaped piece of *C. stigmatosoma*. D, same of *C. quinquefasciatus*

This proposed couplet is based upon a study of more than forty permanently mounted larvae of *C. stigmatosoma* from two miles north and twelve miles north of Fort Davis, Texas; and upon a study of more than fifty mounted larvae of *C. quinquefasciatus*. Three or more larvae of *C. quinquefasciatus* have been examined from each of the following localities in Texas: Abernathy, Austin, Beaumont, Coyote, Eagle Pass, Fort Stockton, Raymondville and Sheffield.

SUMMARY AND CONCLUSIONS

It has been shown that the larvae of *Culex stigmatosoma* Dyar in Texas are extremely variable in the number and arrangement of the hair tufts on the siphon. There is also considerable inconsistency in published descriptions of these same features. The writer has not had an opportunity to examine large series of these larvae from other localities, but it seems possible that the discrepancies in the literature are due to comparable variations in other areas. It would

be of value if workers elsewhere would check this possibility by studying large series of larvae.

It was also found that *C. stigmatosoma* larvae in this area varied in other features that have been used to differentiate this species from similar species. This variation, plus variations in the larvae of *C. quinquefasciatus*, cause the differentiation of these two groups in this area to be more difficult than current keys indicate.

No single easily seen feature has been discovered by which these larvae can be invariably distinguished, but of three characteristics selected, at least two have always been different in the larvae that were studied. These characteristics are the structure of the stirrup-shaped piece, the number of branches in the lateral abdominal hairs of segments III and IV and the number of branches in the sub-dorsal or number 1 hairs of these same segments.

The presence of intergrades or the overlapping of features between two species is often considered as an indication that hybridization may be occurring between the two. It appears unlikely, however, that hybridization is the explanation of the present situation, since no intergradation in adult features has been discovered.

Difficulty has apparently been experienced in some regions in differentiating between the larvae of *C. tarsalis* Coq. and *C. stigmatosoma*. This problem has not been encountered in this area.

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ATLAS DES LARVES D'INSECTES DE FRANCE. NOUVEL ATLAS D'ENTOMOLOGIE, NO. 10, by RENAUD PAULIAN. 1956. N. Boubée & Cie., Paris, France. 222 pp., 80 text figures, 20 plates, paper bound, 1800 francs.

After a short Introduction (dated July 1, 1946) and a general discussion which includes explanations of terms, morphology, etc., the orders are taken up individually. Expressly omitted are the Protura, the Apteryogota and the Paurometabola (Orthoptera, Hemiptera, Psocoptera, etc.). A short systematic table, to separate the orders which are included, is presented on pages 32–33. The arrangement of the orders is apparently an expression of the author's convictions on the natural relationships of the groups. However, it is always rather startling to find the Hymenoptera considered more primitive than the Neuroptera, Mecoptera, Trichoptera, etc. The Strepsiptera are treated as a separate order and discussion of them is confined to a single page (p. 49).

For the most part no keys are presented to sub-ordinal groups. An exception to this is found in the Lepidoptera where a key to the principal families is given (pp. 80–85) that has been adapted from those of Fracker and de Gerasimov.

The treatment of the orders is quite unequal. The discussion of the Hymenoptera is given on 12 pages (pp. 50–61) whereas that of the Coleoptera requires 34 pages (pp. 101–134). A typical example of the arrangement of the subject matter in an order might be that on the Trichoptera (pp. 68–78). A complete diagnosis for larvae of the group is followed by a discussion of biology and types of larval cases. The included families are then treated in sequence, each (except Odontoceridae and Helicopsychidae) with a brief family diagnosis. One to four species are listed in each family and a short description is provided together with a habitus drawing.

Most of the illustrations are included on 20 plates (pp. 160–199). The 4 plates of drawings of lepidopterous larvae (pls. VII-X) are in color, the other drawings are in black and white. Probably some of the figures are original but most of them have been copied from other publications. Occasionally proper credit is given for those copied, as for example text figures 54 to 64 of the last abdominal segments of various elaterid larvae, from Korschefsky. Many of the drawings of coleopterous larvae (pls. XI to XVII) are taken from Böving and Craighead, without comment as to source.

Publications on insect larvae should be encouraged, especially those that contain original and correct information. Interest in immature forms is stimulated in this manner. For that reason I consider the publication of this book justifiable. At the same time we are faced with the need for publications that will make identification of larvae as complete as possible. Paulian has not succeeded in meeting this need. The book will have limited interest to entomologists in North America, either research entomologists or teachers.

W. H. ANDERSON.

A NEW SPECIES OF AEDES (OCHLEROTATUS) FROM TREE HOLES IN SOUTHERN ARIZONA AND A DISCUSSION OF THE VARIPALPUS COMPLEX (DIPTERA: CULICIDAE)[1]

JOHN N. BELKIN AND WILLIAM A. McDONALD
University of California, Los Angeles

In August 1955 the junior author collected a small number of biting females and reared three females and one male of what appeared to be the common western tree-hole mosquito, *Aedes varipalpus* (Coquillett, 1902), in the Santa Rita Mountains, Santa Cruz County, Arizona. Examination of this material by the senior author revealed that this form represents an undescribed species so remarkably similar to *varipalpus* in general facies that it had been reported as the latter species by Dyar (1922:86) from the Santa Catalina Mts. in Arizona. Apparently the same species was found by Ross (1943:86) in Baja California, Mexico. The discovery of this mosquito throws some light on the relationships of *Aedes varipalpus* and suggests that other forms exist in this complex. We are presenting at this time a detailed description of the new species together with a discussion of the complex, in order to call attention to the involved problem, with the full realization that its solution cannot be accomplished without much additional material. The descriptive terminology used is that of Belkin (1953a) except for corrections in the nomenclature of the larval and pupal chaetotaxy (Belkin, 1953b).

We are indebted to Dr. Alan Stone and the U. S. National Museum (USNM) and to Dr. E. S. Ross and the California Academy of Sciences (CAS) for the loan of specimens on which the above-mentioned literature records are based. For additional material we wish to thank Dr. R. M. Bohart, University of California, Davis (UCD), and Dr. G. D. Butler, Jr., University of Arizona, Tucson (Univ. Ariz.). Finally we are grateful to Mr. Charles L. Hogue for the drawings of thoracic scale patterns.

Aedes (Ochlerotatus) monticola

Belkin & McDonald, n. sp.

Aedes varipalpus, Dyar, H. G., 1922. U. S. Nat. Mus., Proc. 62: 86 (In part).
Aedes varipalpus, Ross, E. S., 1943. Pan-Pacific Ent. 19: 86.

DIAGNOSIS

FEMALE.—General facies as in *A. "varipalpus"*, Auct.[2] (as described by Carpenter and La Casse, 1955:257), from which it can immediately be distinguished by the presence of a large postcoxal tuft of white scales, the absence of a basal white ring on the fourth hind tarsal segment, the presence of several lower mesepimeral hairs and more extensive white scaling on the pleura. *Wing* about 2.75–4.0 mm. *Proboscis* dark-scaled, usually a few white scales at base ventrally. *Palpus* about 0.25 of proboscis; dark; apex and two bands white. *Vertex* with median white stripe, submedian dark spots, lateral white patches and ventrolateral dark areas; vertical and occipital erect scales numerous and extensive, white on median stripe, dark on submedian. *Antenna* with dark integument; torus and first flagellar segment each with a median patch of white scales. *Scutum* with dark integument; a broad median stripe of golden scales extending to middle; a pair of patches of golden scales on fossa and anterior supraalar area; anterior and lateral margins with patches and more or less continuous lines of pure white scales; a single median posterior acrostichal and a pair of dorsocentral patches of pure white scales; prescutellar space bordered with white scales; yellowish scales in curved line mesad from scutal angle and straight line caudad from dorsocentral patches; remainder with bronzy scales with purplish reflections. *Scutellum* with large patches of pure white, broad, curved scales. *Pleuron* with large patches of pure white, decumbent, semi-erect or erect scales; *ppn* in addition with a few dark scales; no hypostigial scale patch; large scale tuft on postcoxal area; *lme* with scale patch extending to lower eighth; metameron with small scale patch; bristles and hairs light and numerous, on *ppn* predominantly dark; several light *lme* hairs. *Paratergite* white-scaled. *Wing* dark-scaled except for small patch of white scales at base of C; scales linear on dorsum of R_2+_3, R_2 and R_3, narrow on R_s, M and 1A, broad elsewhere on dorsum; a small patch of dark scales at base of 1A ventrally. *Haltere* light-scaled. *Legs* generally bronzy black with purplish reflections and with pure white areas as below: conspicuous white knee spots; femora speckled with white anteriorly and extensively white posteriorly, with basal and apical white rings; tibiae with broad basal ring and small apical patch of white scales; fore and mid tarsus with broad basal and apical white rings on segment 1, and a broad apical white ring or dorsal patch (fore tarsus) on segment 2; hind tarsus with broad basal and apical

[1]Accepted for publication May 2, 1956.
[2]See "Addendum", p. 191. This species should be called *A. sierrensis* (Ludlow, 1905).

white rings on segment 1, broad apical white rings on 2 and 3, segment 4 usually entirely dark, segment 5 entirely white. *Abdomen* largely bronzy black with purplish reflections on tergites and with posteriorly curved basal bands and connected lateral basal patches of pure white scales; a few apical white scales, more numerous on caudal segments and forming complete apical bands at least on segments 6 and 7; tergite 1 white in middle and laterally; laterotergite white-scaled; sternites predominantly white-scaled on basal segments, and dark-scaled on distal; segment 8 completely retractile.

MALE.—Distinguished from the very similar *A. "varipalpus"*, Auct. by the same characters as the female. Coloration in general as in the female. *Proboscis* with a white-scaled ventral streak to middle and a submedian white ring. *Palpus* slightly longer than proboscis; segment 1 with broad and segment 3 with narrow apical white bands; segment 2 entirely dark; segment 4 with basal white ring and a few apical white scales; segment 5 with broad basal white ring. *Antenna* with penultimate segment about as long as 6 preceding segments, apical about 0.77 of penultimate. *Abdomen* with basal tergal white bands straight and extending all the way to sides; no apical light scales on tergites.

MALE GENITALIA.—Conspicuously different from *A. "varipalpus"*, Auct. (as figured and described in Carpenter & La Casse, 1956:258). Sidepiece without apical lobe; basal lobe small but distinct, bearing one long differentiated apically curved seta and several smaller bristles; no short median bristles as in *"varipalpus."* Clasper about 0.5 sidepiece, swollen before middle, strongly curved on apex; spine about 0.3 clasper length, slender. Claspette stem length 8.0 median width, slightly and uniformly curved; filament about 1.1 stem, cylindrical at base, expanded and flattened beyond middle, apex attenuated and sharply curved.

PUPA.—Generally as in *A. "varipalpus"* from California but readily distinguished by uniform light bright yellowish pigmentation, less distinct integumentary sculpturing, much broader paddle, shorter cercus and details of chaetotaxy. Trumpet uniformly bright yellowish brown; index 5.0; tracheoid distinct. Paddle index 1.4; external margin rather uniformly curved; mesal part wider than outer. Hairs strongly pigmented, mostly single or with a few branches. Abdominal hair 2 on segments III–VII usually external to or in line with hair 1, at least not conspicuously mesal; hair 5 far removed from caudal border on segments IV–VI, strongest hair on these segments; hair 3 strongest hair on segments II and III; hair 7 strongest hair on segment VII; hair 1 single, weak and inconspicuous on segments III–VII. Cercus broad, prominent, with convex external margin.

LARVA.—Very similar to *A. "varipalpus"*, Auct. (as described and figured in Carpenter and La Casse, 1955:258–9), from which it can be distinguished by the shorter and broader antenna, shorter siphon, the absence of hair 11 on abdominal segment I, fewer branches in hairs 1–II–VII, and other details of chaetotaxy. *Antenna* smooth; about 0.4 of head capsule; index about 7.0; hair 1–A at about 0.64 from base, length about 0.5 of antenna. *Head hairs:* 4(7; 5–10), 5(1; occasionally barbed), 6(1; 1–3), 7(4; 3–7). *Prothoracic hairs:* 1(2, 3), 2(1), 3(2), 4(2; 1–2), 5(2; 2–3), 6(1), 7(3, 2, 4). *Abdominal hairs:* 11–1 absent, 6–I–III(2; rarely 3), 6–IV, V(2), 6–VI(1, 2), 1–I(3–6), 1–II–VII(2, 3; 2–4). *Comb scales* (10–20) in a single, partial double or triple irregular row; individual scale in middle of caudal row narrow, elongate, more or less parallel-sided to apex, with lateral and apical fringes. *Siphon:* index about 3.0 or less; strong concavity on basal ventral surface; pigmentation very dark, sculpturing indistinct; pecten teeth (9–16) evenly spaced in basal 0.3, individual tooth with subbasal spines; hair 1(3) in basal 0.4, not distinctly barbed. *Anal segment:* saddle brown, extending only about halfway around segment; hair 1(2; 1–4; branches uneven, longest about 3.2 saddle length), 2(3; 2–3; about 3.6–5.2 saddle length), 3(1; about 6.0 saddle length), 4a–d on grid, 4a(1), 4b(1; 1–2), 4c(2, 1), 4d(2–4), 4e(2; 2–3), 4f(1; 1–3); anal gills subequal, about 3.5 saddle length.

DESCRIPTION

FEMALE.—Wing: 2.75–4.0 mm. Abdomen: 1.9–3.0 mm. Proboscis (labium and labella): 1.75–2.4 mm. Front femur: 1.3–2.0 mm. Specimens examined: 22.

Head: Integument black throughout (dark brown in old specimens); eyes rather widely separated on the frons. Vertex (occiput in terminology of Carpenter and La Casse, 1955) with a median patch of rather broad, apically pointed, curved, pure white, decumbent scales, arranged on edge and appearing linear from above, continued as a dense patch on frons below; a narrow orbital line of short, broad, flat, pure white scales, expanding into a patch of similar but larger decumbent scales laterad, no orbital line of scales below this patch; a pair of submedian dark areas with sparse scaling, anteriorly scales are narrow, curved, decumbent, rather dense and bronzy black with purplish iridescence, posteriorly scales are pure white, linear, decumbent and rather numerous on occiput where they join the median white patch and longer, broader, curved, semi-erect and scattered toward middle of vertex; postgena with a patch of short, broad, flat bronzy black scales below lateral white patch of vertex, followed by similar pure white scales below; erect forked scales of vertex numerous and rather short, white on median patch, black

on submedian dark areas, rather uniformly distributed from occiput to orbital bristles except for anteromedian triangular area from which they are absent; 2 or 3 pairs of light orbital bristles on white median patch of vertex, median pair developed as frontals; 4 dark orbital bristles near anterior border of each submedian dark area of vertex, bristles far removed from orbital border; 5 dark orbital bristles on each postgenal area with at least 1 additional bristle below. Clypeus bare. Palpus about 0.25 of proboscis; all segments without scales on ventral surface; palpifer completely without scales; segment 1 immovably joined to palpifer, with dark scales and usually at least a few white ones on apex; segments 2 and 3 united, dark-scaled at base and with a large apical white band; segment 4 (long segment) dark-scaled at base and white on apical third, a few scattered white scales also present before apex; segment 5 minute, not visible except in slide preparations, hidden by white scales of 4; dark scales bronzy black with purplish reflections, white scales pure white; bristles short, dark. Labium uniformly and smoothly covered with small bronzy black scales with purplish iridescence, usually at least a few pure white or whitish scales ventrally near basal bristles, often a conspicuous patch of such scales, occasionally a few white or whitish scales elsewhere on ventral surface; labella with dark integument and a few small dark scales at base dorsally. Antenna slightly shorter than proboscis; integument black or dark brown throughout except for lighter membranes; torus (pedicel) with conspicuous patch of small pure white, broad scales on mesal surface, particularly toward dorsal apex; mesal surface of first flagellar segment with a conspicuous patch of similar scales; about 6 long bristles in each flagellar whorl; last three flagellar segments subequal to each other and to more basal segments.

Thorax: Scutal integument uniformly black or dark brown except for lighter postalar margin and scutal angle; a striking but rather variable scale pattern, usually as in Figure 1a; scales of three general colors; dark scales bronzy black with purplish iridescence, occupying most of the dark areas in the figure except for bare prescutellar space but most conspicuous on anterior part of fossa, posterior dorsocentral and prescutellar line where they are rather short, broadened, curved and decumbent, and in the supraalar area where they are long, linear, straight and appressed; light scales pure white or golden, with some intermediate yellowish ones; white scales generally broad, apically pointed, curved and decumbent but more linear, straighter, appressed ones also present; anterior promontory and humeral angles with conspicuous patches of broad white scales in line respectively with acrostichal and dorsocentral bristles; from humeral patches a more or less continuous curved lateral line of white scales to scutal angle where a patch of white scales is formed; a conspicuous patch of narrow white scales just laterad of dorsocentral line at level of paratergite, this patch (dorsocentral) more or less connected to scutal angle patch by whitish or yellowish scales; a patch of white scales on antealar space, scales largely outstanding, some linear, others broadened and curved, a few broad white scales extending into supraalar area; a conspicuous median patch of broad white scales at caudal end of acrostichal line in front of

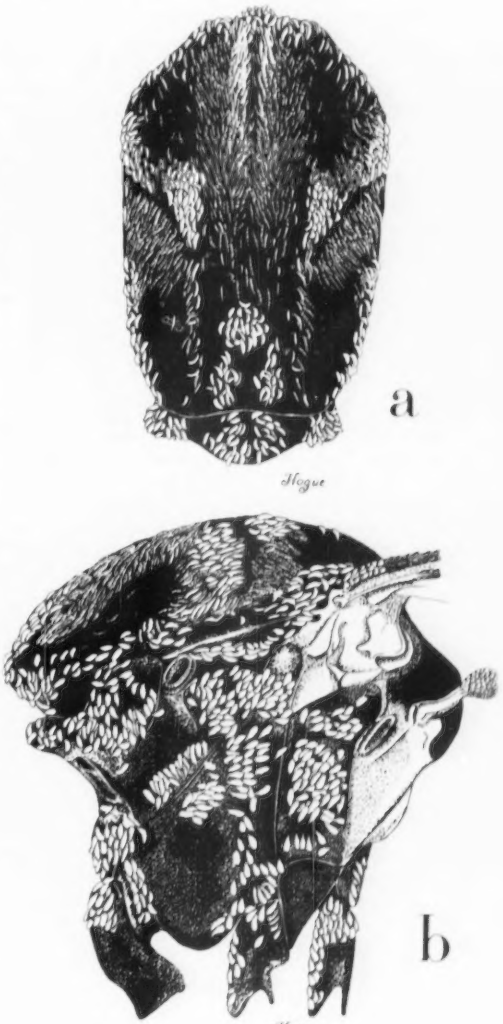


FIG. 1. Thoracic scale ornamentation of adult female of *Aedes monticola*, n. sp.; bristles not shown. *a*, Dorsal aspect of mesothorax. *b*, Left lateral aspect of entire thorax.

prescutellar space; lateral borders of prescutellar bare space, mesad of prescutellar lines, with broad curved white scales, these mesoprescutellar lines often not extending all the way cephalad; a narrow interrupted line of broad whitish or yellowish scales laterad of posterior dorsocentrals and prescutellars, more or less connected to dorsocentral patch and often abbreviated caudally; golden scales generally narrower and straighter than white ones, forming a large conspicuous anterior median stripe between dorsocentral bristles from cephalic margin of scutum to slightly beyond its middle, usually a number of white or whitish scales on anterior portion of this stripe extending from anterior acrostichal white scale patch; a small patch of golden scales in posterior part of fossa; a conspicuous and characteristic patch of golden scales between dorsocentrals and supraalars at level of paratergite; acrostichal bristles well developed, light on anterior promontory and for a short distance caudad where white scales predominate, remainder dark; dorsocentrals larger than acrostichals, light on humeral angles, remainder dark, merging caudad with dark prescutellars; a more or less regular row of strong dark presuturals extending laterocaudad from humerals; usually 2 to 4 dark transverse bristles between presuturals and dorsocentrals near caudal end of fossa; supraalars dark, strongly developed. Scutellum dark brown; lobes with conspicuous patches of broad, pointed, curved decumbent pure white scales; each lobe with about 6 long dark bristles and 4 or more smaller lighter bristles. Postnotum dark brown. Pleural integument brown to dark brown, lighter around anterior spiracle, under wing base, entire metapleuron and membranous areas; vestiture of pure white scales as in Figure 1b, scales predominantly decumbent, semi-erect or erect and at least slightly curved, giving a shaggy appearance to pleuron, all scales broad, some rounded apically, others pointed; in addition some dark scales similar to those on scutum present on upper part of *ppn* in the white-scaled patch and cephalad; *apn* with 9 or 10 dark bristles on upper part and with 1 or more dark bristles and up to 20 light hairs and bristles on lower part; *ppn* with about 6 dark bristles in a more or less regular row, caudad or ventrad of which usually 4 or more light bristles, 1 or 2 light or dark bristles some distance cephalad on disc of *ppn*; *ppl* (propleuron, proepisternum) with 20 or more light bristles and hairs; *pst* (prosternum) with a pair of large lateral white-scaled patches, continuous with patches of *ppl*, some of the scales appear to arise in membrane anterior to fore coxa, others from lateral margin of *pst*, which also bears 4 or more light hairs; *pcx* (postcoxal area, membrane caudad of fore coxa) with a very conspicuous tuft of elongate white scales; *ptr* (paratergite) with semi-erect and erect scales along outer border and below; *psp* (postspiracular area) with 10 or more light hairs, semi-erect curved scales among hairs, a separate patch of flat scales above; *ssp* (subspiracular area) with long patch of appressed scales; *pra* (prealar area) with about 20 light hairs and 1 or 2 linear erect scales on knob, a patch of appressed scales below knob; *stp* (sternopleuron) with bristles and hairs all light, a more or less regular longitudinal row of about 6 bristles at upper end of scale patch followed ventrally by numerous scattered light hairs on caudal third or fourth of sclerite down to lower margin, scales in this area erect, at middle just below transverse row of bristles scales are semi-erect and reach anterior border of sclerite, this patch is continued ventrocephalad parallel to anterior margin but not reaching it, scales in this area decumbent; *ume* (upper mesepimeral area) with 30 or more light hairs and small patch of erect scales below hairs; *lme* (lower mesepimeral area) with a very extensive patch of scales continued at least to lower eighth of sclerite, cephalodorsal part of patch ruffled and usually with 4 or 5 small light *lme* hairs, caudal part of patch with scales decumbent; *mtm* (metameron, metasternum) with small flat patch of scales; all other sclerites and membranes without scales, bristles or hairs. Haltere yellowish at base and stem, white-scaled on upper part of stem and knob.

Wing: Venation normal; distance between crossveins slightly more than length of *m-cu*. Scales all dark, except for small patch of white scales at extreme base of C and occasional light scales at base of R; scales linear on dorsal surface of R_{2+3}, R_2 and R_3, narrow on R_s (except at base) and 1A (except at base), broad on dorsal surface of other veins; a small but conspicuous patch of dark scales on base of 1A on ventral surface; bristles on stem vein (base of R) very short, barely projecting beyond scales. Fringe rather light on posterior border, whitish near base; border scales of fringe and alula dark.

Legs: Coxae and trochanters with brown or dark brown integument, membranes lighter. Fore coxa with decumbent pure white scales covering anteromesal and anterior surfaces except for a variable number of dark scales near middle, scaled area bounded laterally by an irregular row of dark bristles from middle of anterior margin of coxa down along anterior margin of articular membrane of trochanter to mesal surface; numerous scattered light bristles and hairs basad of dark bristles and on scale patch mesad; outer surface largely bare, white scales in small tuft at base continuous with anteromesal scales, a small white, decumbent scale patch on apical third (free portion of coxa) preceding a row of short bristles and a few dark and white narrow scales along posterior margin of trochanterial membrane to prosteroapical margin of coxa. Mid coxa with white scaling on anteromesal surface as on fore coxa except that it extends on to outer surface so that the more regular, dark and light boundary

bristles are about one-third from anterior margin; dark scales absent; light bristles and hairs restricted to base; outer surface with only the basal tuft of white scales; posterior margin bare except for a few elongate dark scales and very small bristles. Hind coxa with a dense patch of white decumbent scales at base on outer surface, extending slightly to anterior surface and prolonged ventrally as an irregular streak along anterior margin to apex, near middle of streak a triangular projection of streak to anterior surface; a few short dark bristles on apex of anterior margin; a large bare space on outer surface caudad of scale streak followed by dark and light boundary bristles which are near posterior margin; posterior surface with a few light hairs, mostly in basal half, and a few light scales at base. Trochanters with mixed white and dark, small, appressed scales except for bare external (dorsal) articular surface. Femora all with narrow basal and apical rings; all largely dark-scaled on dorsal margin (fore femur speckled with white); anterior surface of fore femur largely dark-scaled, a narrow, irregular white streak from base to near apex along ventral margin; mid and hind femora dark-scaled on anterior surface and ventral margin but speckled with white scales which are more numerous proximad; posterior surface of fore femur largely white-scaled, speckled with dark scales which are more numerous distad; mid and hind femora with posterior surfaces largely white-scaled in basal half, dark-scaled distad but with some white scales, more on mid femur; dark scales bronzy black with purplish reflections, white scales pure white; bristles largely white. Tibiae dark-scaled except for broad basal ring and small apical patch of white scales; scale and bristle coloration as on femora. Fore tarsus with broad basal and apical white rings on segment 1, a broader white apical dorsal patch on segment 2, remainder dark-scaled; mid tarsus similar to fore tarsus except that on segment 2 dorsal patch is now an incomplete or complete ring and segment 5 is somewhat lighter; hind tarsus with very broad basal and apical white rings on segment 1 (white scaling occupying almost half of segment), segments 2 and 3 with broad apical rings (white scaling occupying about one-third of segments), segment 4 entirely dark-scaled or rarely with a few white scales dorsally near base or apex, segment 5 entirely white-scaled, remainder dark-scaled; scale and bristle coloration as on femora. Fore claws subequal, slightly curved, with slender tooth in basal third; mid claws subequal, somewhat larger, more curved, with slender tooth in basal third; hind claws subequal, simple.

Abdomen: Tergite 1 sparsely scaled, central area with variable number of white scales in the middle and dark scales on the sides, lateral areas with scattered white scales, laterotergite with dense patch of white scales; tergites 2–6 densely covered with bronzy black scales with purplish reflections, pure white scales forming rather narrow, curved basal bands which are narrowed laterad and joined to large lateral white-scaled, square or rectangular, basal patches, a few apical white scales on 2, becoming more numerous on caudal segments and forming a complete band on at least tergite 6, sometimes also 5; tergite 7 without distinct dorsal basal band but with lateral basal patches and a broad apical white band; tergite 8 completely retractile. Sternite 2 white-scaled; sternites 3 and 4 largely white-scaled and with apicolateral dark patches, 3 sometimes completely white; 5 and 6 with more extensive subapical dark patches, on 6 these form a continuous subapical dark band followed by narrow apical light band; 7 white in the middle, dark laterad; extent of dark and light scaling extremely variable. Abdominal bristles numerous, small, all light.

MALE (173-104, *Holotype*).—Wing (exclusive of fringe): 3.1 mm. Proboscis: 2.2 mm. Front Femur: 1.7 mm.

Generally very similar to female. A white-scaled streak covering entire ventral basal 0.48 of proboscis and extending dorsad to form a white ring at 0.33 to 0.48 of proboscis. Palpus slightly longer than proboscis; scaling bronzy black with purplish reflections and pure white; segments 1–3 unscaled ventrally, 4 and 5 completely scaled; segment 1 with broad dorsal apical white band; segment 2 entirely dark; segment 3 not articulated with 2, widened apically and curved up, a few short hairs subapically, a narrow dorsal apical white band; segments 4 and 5 broadened, turned down, with a small number of long dark bristles; segment 4 with basal white ring and scattered white scales apically; segment 5 with broad basal white ring. Antenna slightly shorter than proboscis; basal flagellar segments short and wide; penultimate very slender, about as long as 6 preceding segments, with dense whorl of bristles near base and sparse short hairs beyond; apical slender, about 0.77 of penultimate, with whorl of 6 bristles near base and with numerous short hairs beyond; flagellar whorls with 30 or more long bristles projecting mostly dorsad and ventrad, longest bristles about 0.5 of flagellar length. Fore and mid claws enlarged, unequal, slender, moderately curved, all with slender subbasal tooth; hind claws subequal, simple. Abdominal tergite 1 without white scales in center; segments 2–5 with basal white bands straight, basal lateral white spots absent but bands a little wider at these points; no apical light scales or bands; other segments not seen. Abdominal sternite 2 as in female; 3 and 4 white-scaled and with apical lateral dark patches; 5 with basal lateral white patches, remainder dark-scaled other segments not seen.

MALE GENITALIA (173-104, *Holotype*).—As in Figure 2a. Ninth tergite displaced in

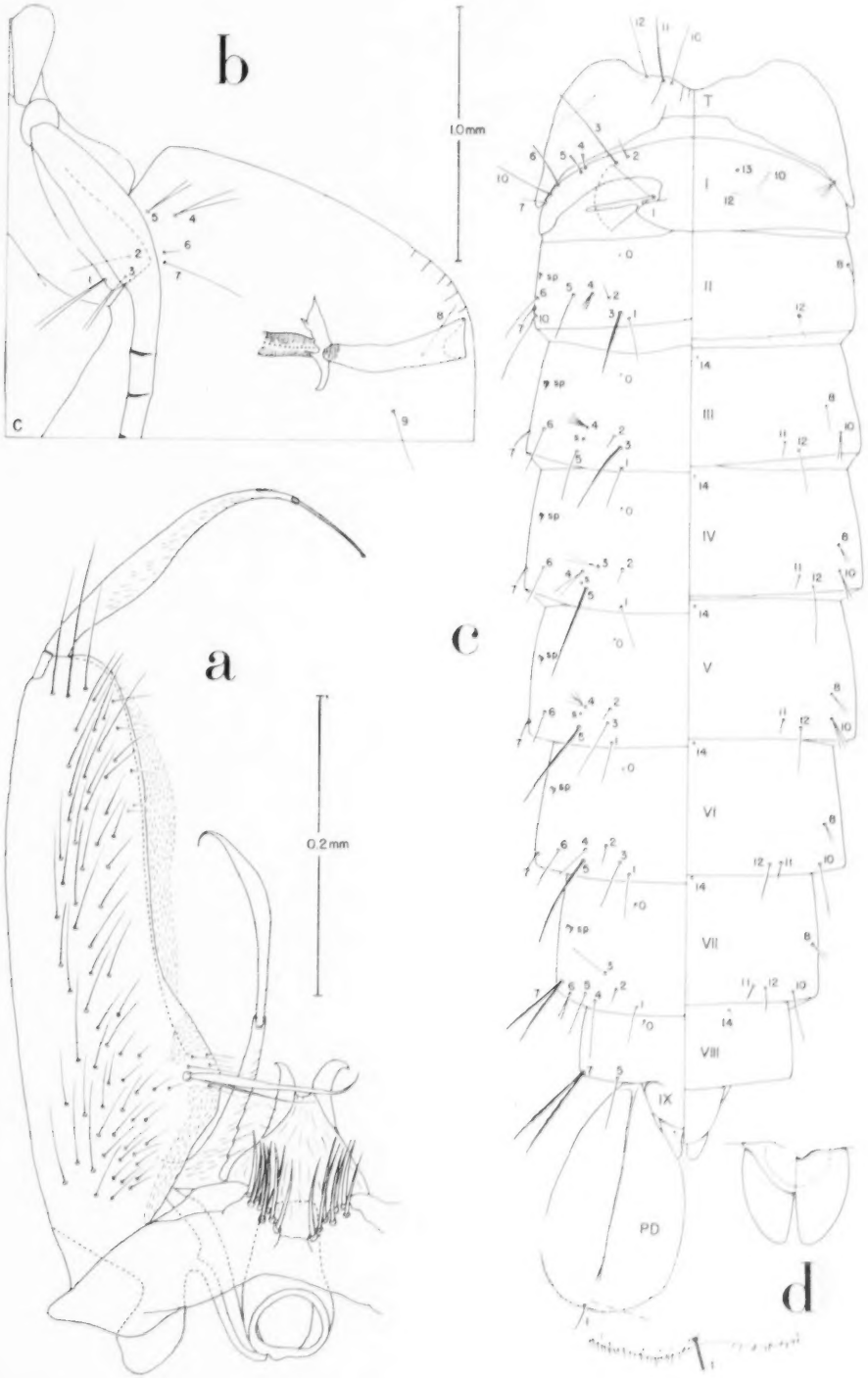
mount, interlobar space apparently less than width of one lobe; lobe broader than long, bearing 7 or 8 spines. Proctiger with tenth sternite strongly sclerotized and heavily pigmented on hooked apical portion, 3 small setae laterally; tenth tergite unsclerotized. Sidepiece (basistyle) about 4.0 as long as width at middle, uniformly spiculate including mesal membrane; mesal two-thirds of dorsal surface with short slender hairs at base, hairs longer and heavier toward apex, mesal dorsal margin bare except for a few short slender hairs on apical 0.25; outer third of dorsal surface, external and ventral surfaces with usual strong bristles and scales, mesal ventral margin with moderately heavy, long bristles from base to apex. Apical lobe completely absent. Basal lobe moderately prominent, separated by distal and basal membranes; dorsal surface with one long, strong differentiated seta with strongly curved apex, projecting mesad and followed ventrally by one or more strong attenuated bristles; remainder of apex with numerous short, weak hairs, more numerous and prominent on ventral surface. Clasper (dististyle) including claw slightly more than 0.5 of sidepiece; swollen before middle, apex strongly curved; spiculate on mesal and dorsal surfaces except at base, one to three small setae near apex; spine (claw) slightly less than third of clasper body, deeply inserted on apex, very slender, nearly straight and uniform in width, apex slightly attenuated, apical membrane very small. Claspette stem very slightly and uniformly curved, gradually and slightly narrowed apically, length about 8.0 median width; dorsal mesal surface very sparsely short-spiculate, ventral mesal more extensively long-spiculate, 4 to 6 short setae on ventral mesal margin near base; filament about 1.1 stem, cylindrical at base, strongly expanded and flattened beyond middle, gradually attenuated and then sharply recurved at apex. Mesosome simple; length about 2.0 basal width in dorsal aspect; poorly sclerotized except at base, dorsally and laterally; apex broad, smooth, simple, slightly truncate. Other structures not clear in mount.

PUPA.—Abdomen: 3.4(♂)–4.0(♀) mm. Trumpet: 0.55(♂)–0.65(♀) mm. Paddle: 0.92(♂)–1.0(♀) mm. Specimens examined: 2♂, 3♀.

Cephalothorax (Figure 2b, c): Uniformly light yellowish except for narrow blackish middorsal area on caudal part of mesonotum; metanotum darkened except laterally. Trumpet uniformly bright yellowish brown except for blackish tracheoid; length about 5.0 median width; gradually widened from tracheoid to apex; inner wall distinctly separated except in pinna; tracheoid extending to about 0.15–0.20; reticulate with regular imbrications, without conspicuous spicules; pinna about 0.25. All hairs darkly pigmented but small and inconspicuous, simple; relative position, length, and degree of development as figured. Hairs: 1(2, 1; 1–3), 2(1; 1–2), 3(2, 1), 4(2; 1–2), 5(2; 2–5), 6(1, 2), 7(1, 2; 1–3), 8(1; 1–2), 9(1; duplicated in one specimen), 10(1; 1–2), 11(1), 12(1; 1–2). Two additional dorsal hairs, one near 12, the other laterally, occasionally present; a stellate tuft present in one specimen at base of haltere case (possibly homolog of 13T of larva); a small branched hair present in one specimen underneath mesothoracic wing case.

Abdomen (Figure 2c): Uniformly light bright yellowish brown except for blackish anterior median dorsal portion of segment I and darkened intersegmental areas (including middle of anterior border of tergites and sternites 2–7); integumentary reticulation and spiculation inconspicuous. Hairs darkly pigmented, strongly contrasting with integument; relative position, length and degree of development as figured; hair 1 weak, generally single, except on segments I and II; hair 2 on segments III–VII external to hair 1 except on III, where it is usually in line with 1; hairs simple and generally unbranched except as noted, when branched usually some distance from base. Segment I: hair 1 (dendritic tuft; usually 32–34d; one 62–68), 2(1, 2), 4(2; 1–2), 5(2–4; 2–7), 6(1, 2; 1–5), 10(1, 2; 1–3); three additional hairs occasionally present on sternite, a mesal peg, a long hair at level of 1, and a shorter (sometimes branched) hair laterad of level of 1, probably homologs of 13, 12 and 10 respectively of the larva. Segment II: hair 0(1; 1–2), 1(1, 2), 3(1; 1–2), 4(3, 4; 3–5), 5(1; 1–3), 6(1; 1–2), 8(1; 1–2); hair 12 occasionally present. Segment III: hair 0(1; 1–2), 4(2–4), 5(1; 1–2), 6(1; 1–2), 8(1, 2; 1–3), 10(2, 3; 2–4), 14(1–2); dorsal sensillum variable in position. Segment IV: hair 3(2), 4(2, 3; 1–3), 6(1; 1–2), 8(2,1), 10(2; 1–3); dorsal sensillum variable in position. Segment V: hair 4(4, 3; 3–6), 8(2; 1–2), 10(3, 2; 1–4), 14(1; 1–2); dorsal sensillum variable in position. Segment VI: hair 2(1; 1–2), 6(1; 1–2), 8(2, 1). Segment VII: hair 2(1; 1–2), 4(1; 1–3), 6(1, 2), 7(1; 1–2), 8(1–3), 14(1; 1–2). Segment VIII: hair 7(2, 3), 14(1; 1–2). Segment IX: extending to about 0.22 of paddle; hair 1 absent. Paddle as figured: uniformly pigmented a light bright yellowish brown except for darker brown midrib and base of external buttress, basal pigment bar present or absent; length about 1.4 median width; midrib strongly sclerotized, evanescent apically; external buttress indistinct; external margin serrations starting at about 0.5, more prominent

FIG. 2. Male and pupa of *Aedes monticola*, n. sp. *a*, Dorsal (morphologically) aspect of male genitalia, large lateral setae and scales of sidepiece not shown. *b*, Ventral aspect of left anterior portion of cephalothorax. *c*, Dorsal (left) and ventral (right) aspect of metanotum and abdomen of female pupa, with enlarged detail of apex of paddle. *d*, Same aspect of apex of abdomen of male pupa.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apically, arranged in two or three incomplete dorsal rows on apex, teeth slender, sharply pointed; internal margin with similar serrations; apex of paddle emerginate in area of hair 1, usually few or no serrations in this notch mesad of hair 1; hair 1(1, 2; 1–4). Female genital lobe (Figure 2c) extending to slightly less than 0.3 of paddle, without integumentary spiculation; anal segment not separated from genital lobe; cercal sclerites prominent, broad, with convex external margin, extending slightly beyond apex of genital lobe (in one specimen each cercus with a long simple hair). Male genital lobe (Figure 2d) extending to slightly less than 0.5 of paddle, without integumentary spiculations, apex of sidepiece broadly rounded; anal segment almost as long as segment IX, about 0.55 as wide as latter at base.

LARVA.—Head: 0.8–0.95 mm. Siphon: 0.8–0.95 mm. Anal saddle: about 0.28 mm. Specimens examined: 5.

Head (Figure 3b): Width about 1.1 of length; integument light yellowish brown, darkened anteriorly and posteriorly; ocular areas lighter; sculpturing indistinct. Labrum very short, anterior margin very slightly convex. Mental plate small, with 14 to 20 teeth. Maxillary sutures conspicuous, strongly divergent, continued dorsolaterad beyond posterior tentorial pits. Hairs of head capsule strongly pigmented, conspicuous; relative position, length and degree of development as figured. Hair 0 (not seen), 1 (1; occasionally split on apex, quite heavy), 2 (absent), 3 (1; slender), 4(7; 5–10), 5(1; occasionally barbed), 6(1; 1–3), 7(4; 3–7), 8(1; 1–2), 9(2; 1–2), 10(2; 1–2), 11(5–13), 12(1), 13(2, 3; 1–4), 14(1), 15(2–5). Antenna about 0.4 of head, slightly curved and gradually and slightly narrowed to apex, length about 7.0 or less median width; uniformly light yellowish brown; shaft smooth, spicules not developed. Antennal hairs with same pigmentation as shaft except for transparent apex of 5-A; all unbranched; 1-A at about 0.64 from base, length about 0.5 of antenna; basal sense organ very inconspicuous, in basal membrane.

Thorax (Figure 3b): All hairs very strongly pigmented; tubercles brown, present only at base of hairs 9–12-P, 5-M, 6–7-M, 8-M, 9–12-M, 7-T, 9–12-T; apices of long hairs attenuated; long hairs with short barbs largely near base; relative position, length and degree of development as figured. Prothorax: hair 0(7, 6; 5–8), 1(2, 3), 2(1), 3(2), 4(2; 1–2), 5(2; 2–3), 6(1), 7(2–4), 8(5, 6; 5–7), 9(1, 2), 10(1), 11(2, 3; 1–3), 12(1), 14(2; 2–3). Mesothorax: hair 1(2), 2(1; 1–2), 3(1), 4(1, 2), 5(1; 1–2), 6(3; 3–5), 7(1), 8(7, 6; 5–7), 9(4–6), 10(1), 11(1, 2), 12(1), 13(5–11), 14(3–7). Metathorax: hair 1(3–4; 2–4), 2(1), 3(3, 2), 4(3, 4; 2–4), 5(1; 1–2), 6(1), 7(6–8; 6–9), 8(4–8), 9(4; 4–6), 10(1), 11(1), 12(1), 14(4–6; 4–7).

Abdomen (Figure 3b, c): Hairs strongly pigmented; tubercles absent except 2–3-X; long hairs without barbs except a few on those of caudal segments; relative position, length and degree of development as figured. Hair 11-I absent. Segment I: hair 1(3–6), 2(2), 3(; 1–2), 4(3–5), 5(2–3; 2–4), 6(2), 7(1), 8(2; 1–2), 10(2), 12(1), 13(2). Segment II: hair 0(1), 1(2–4), 2(1), 3(3–5), 4(1), 5(2, 3; 1–3), 6(2; 2–3), 7(2, 1), 8(2; 1–2); 9–12(1), 13(2–4; 2–5). Segment III: hair 0(1), 1(2; 2–3), 2(1), 3(2; 1–3), 4(1), 5(2, 3; 1–3), 6(2; 2–3), 7(2–3), 8(1; 1–2), 9, 10(1), 11(1, 2), 12(1), 13(2), 14(1), dorsal sensillum present. Segment IV: hair 0(1), 1(2, 3; 2–4), 2(1; 1–2), 3(2, 1; 1–3), 4(1), 5(2, 3), 6(2), 7(3, 2), 8–10(1), 11(1, 2), 12(1), 13(2, 3), 14(1), dorsal sensillum present. Segment V: hair 0(1), 1(2; 2–4), 2(1), 3(2; 2–3), 4(1), 5(2; 2–3), 6(2), 7(2; 2–3), 8(1; 1–2), 9–12(1), 13(2; 2–3), 14(1), dorsal sensillum present. Segment VI: hair 0(1), 1(3, 2), 2–4(1), 5(2; 2–3), 6(1, 2), 7(1), 8(2, 1; 1–3), 9, 10(1), 11(1; 1–2), 12(1), 13(7–9; 5–10), 14(1). Segment VII (Figure 3d): hair 0(1), 1(2–3), 2(1; 1–2), 3(1, 2), 4(1), 5(2), 6(4, 3; 3–5), 7(1), 8(4–6), 9(1, 2; 1–3), 10–12(1), 13(2; 1–3), 14(1). Segment VIII (Figure 3d): comb scales (10–20) in a single, partial double or triple irregular row; those of caudal narrow, elongate, more or less parallel-sided to apex, with lateral and apical fringes; more proximal ones pointed apically and with fringe reduced; hair 0(1), 1(3; 2–3), 2(1), 3(3; 2–5), 4(1), 5(4; 3–5), 14(1). Siphon: as in Figure 3d; length (on dorsal surface) about 3.0 or less median width; characteristic concavity on basal ventral surface and slight submedian swelling; pigmentation uniformly deep blackish brown, sculpturing indistinct; acus strongly developed; pecten extending to about 0.3 of ventral length; teeth (9–16) very dark except for light acute apex, with 2 or more subbasal spines; valves as figured, more lightly and less uniformly pigmented than siphon; hair 1(3), 2–7(1), 8(1–3), 9(1), others not studied. Segment X (Fig. 3d): saddle extending only about halfway around segment, brown except for darker dorsal surface, sculpturing indistinct except apically; gills subequal, broad, sausage-shaped, apically rounded, length about 4.3 median width and about 3.5 saddle length; hair 1(2; 1–4; branches uneven, longest about 3.2 saddle length), 2(3; 2–3; about 3.6–5.2 saddle length), 3(1; about 6.0 saddle length), 4a(1; about 5.3 saddle length), 4b(1; 1–2; about 6.0 saddle length), 4c(2, 1; about 3.7 saddle length), 4d(2–4; about 1.5 saddle length), 4e(2; 2–3; about 0.9 saddle length), 4f(1; 1–3; about 0.5 saddle length); 4d and f basad of grid; no sclerotization laterad of grid.

TYPES

Holotype ♂ (UCLA 173-104) with associated larval and pupal exuviae and genitalia slide,

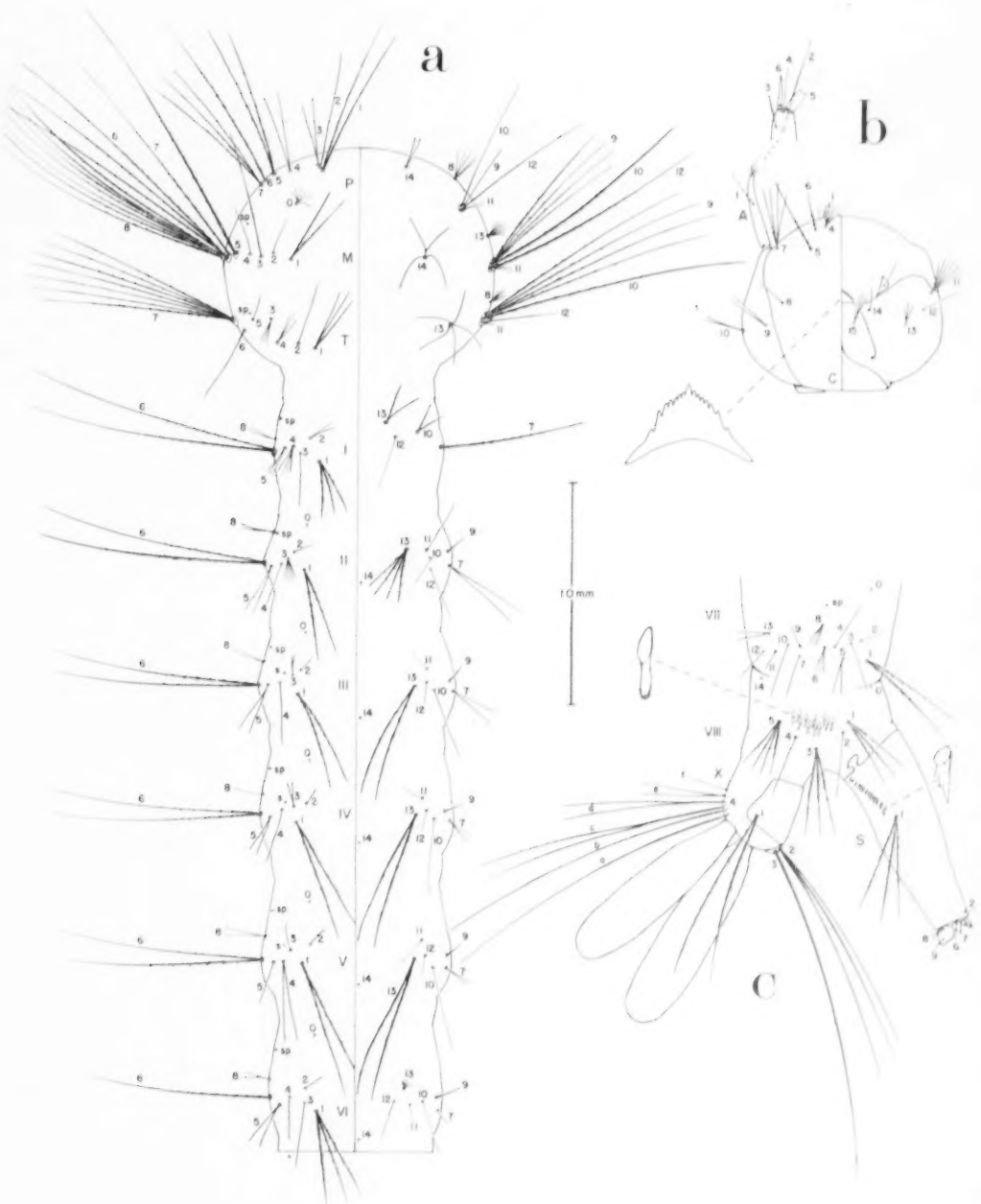

FIG. 3. Fourth instar larva of *Aedes monticola*, n. sp. *a*, Dorsal (left) and ventral (right) aspect of thorax and proximal abdominal segments. *b*, Same aspect of head, with enlarged details of apex of left antenna and of mental plate. *c*, Left lateral aspect of terminal abdominal segments.

Madera Canyon, Santa Cruz County, Arizona, elevation *ca.* 5,000 feet, Aug. 18, 1955, reared from larva collected in rot hole in dead, fallen sycamore trunk (W. A. McDonald) [USNM No. 63244].

Allotype ♀ (UCLA 173-105) with associated pupal exuviae, same data as holotype [USNM].

Paratypes: 2 ♀ (UCLA 173-102, 103) with associated larval and pupal exuviae, 1 whole larva (UCLA 173-101), 1 pupal skin (173-1), same data as holotype; 1 whole larva (UCLA 173-205), same locality, collector and date as holotype but from rot hole in living oak; 14 ♀ (UCLA 170-3), same locality and collector as holotype but collected biting or resting, Aug. 17, 1955 [BMNH, CAS, UCLA]; 1 ♀, Sabino Basin, Santa Catalina Mts., Pima County, Arizona, Aug. 24 (1918) (C. H. T. Townsend) [USNM]; 1 ♀, White House Canyon (Madera Canyon), Santa Rita Mts., Arizona, May 31, 1946 (R. M. Bohart) [UCD]; 1 ♀, Mt. Graham, Pinaleno Mts., Graham Co., Arizona, elev. 7000 feet, July 4, 1940 (S. L. Green) [Univ. Ariz.]; 1 ♀, Santa Rita Mts., Arizona, Oct. 20, 1940 (R. A. Flock) [Univ. Ariz.]; 1 ♀, Chiricahua Mts., Cochise Co., Arizona, June 17, 1937 (R. S. Beal) [Univ. Ariz.].

DISTRIBUTION

ARIZONA. *Graham Co.*: Mt. Graham, Pinaleno Mts. *Cochise Co.*: Chiricahua Mts. *Pima Co.*: Sabino Basin, Santa Catalina Mts. *Santa Cruz Co.*: Santa Rita Mts. (For data see section on Types.)

MEXICO. *Baja California*: La Laguna, Sierra Laguna (Cape Region), elev. 6,200 feet, 2 ♀ biting in pine oak forest (E. S. Ross and G. E. Bohart) [CAS].

VARIATION

The females from Madera Canyon are quite uniform in size and conspicuously larger than the average *A.* "*varipalpus*". However, the other females from Arizona exhibit a variation in size comparable to that found in "*varipalpus*." In the Arizona females the mesonotal ornamentation is extremely variable both as regards the coloration and the shape of scales; even the more or less characteristic supraalar golden patch may be practically entirely replaced by dark scales. On the other hand, the broad anterior median golden stripe is quite constant in our small series. The extent of the white scaling as well as the shape of the scales is also quite variable on the pleura and on the head as is also the white speckling of the femora and the white scaling of the abdomen. In its diagnostic characters of the presence of the postcoxal scale patch and the absence of a white ring at the base of hind tarsal segment 4, ***monticola*** shows absolute separation from "*varipalpus*", Auct.

The two females from Baja California may represent a distinct subspecies of ***monticola*** but the recognition of a separate taxon must await much additional material of all stages. These females possess the postcoxal scale patch and lack the white ring at the base of hind tarsal segment 4 as in the typical ***monticola*** but in other characters of ornamentation they are intermediate between them and "***varipalpus***", Auct. In particular, the anterior median mesonotal golden patch is broken into three lines separated by dark scales and there are fewer and smaller white scales at the anterior end, the supraalar golden patch is apparently lacking, the pleural scaling is not as extensive and there are apparently no lower mesepimeral bristles.

The small number of larvae and pupae, five of each stage, is hardly adequate to evaluate the variability of these stages. However, the diagnostic characters appear to be quite constant. In the pupa there is a remarkably large number of anomalous retentions of larval hairs: four different hairs on the cephalothorax, three on abdominal segment I, one on segment II, and one on the cercus.

BIONOMICS

The immature stages of ***monticola*** have been collected only twice in the same general locality in Madera Canyon in the Santa Rita Mts., Arizona, at an elevation of about 5,000 feet (McDonald, 1956a). In both cases *A. muelleri* Dyar, 1920 was associated with ***monticola***. One collection came from a rot hole in a dead prone sycamore trunk (***monticola*** predominating) and the other from a rot hole in a living oak (***muelleri*** predominating).

Females of ***monticola*** bite man readily. Several females were captured while attempting to feed during the late afternoon of August 17, 1955 in the immediate vicinity of the sycamore log mentioned above. Two of these were successfully blooded on the forehead and cheek of the junior author, but died later in the laboratory without producing eggs. Others were found on the inner sides of the rot hole in the sycamore trunk and among the foliage of the still-living stump of the same tree. A single female was attracted to a Coleman lantern set on a white sheet draped over a nearby log during the night of August 19, 1955 (air temperature 21.5°C.; moon two days old). No males were collected in the field.

Ross (1943:86) reported females of ***monticola*** (as ***varipalpus***) biting in a pine-oak forest at an elevation of 6,200 feet on Oct. 14, 1941 at La Laguna, Sierra Laguna (Cape Region), Baja California, Mexico. This locality is "characterized by its Upper Sonoran biota isolated by several hundred miles of desert from comparable environments in the San Pedro Martir Mountains of the north."

No data are available for the other collections from Arizona except for dates and the fact that all were made in mountain areas. Undoubtedly

the females were collected while attempting to feed.

DISCUSSION

Despite its considerable economic importance, the western tree-hole mosquito has never been the object of an intensive taxonomic study. Coquillett (1902: 292–3) described *Culex varipalpus* from a single female collected by H. S. Barber at Williams, Arizona. Apparently no additional topotypic material has ever been obtained. In 1904 Coquillett identified males, females and larvae reported by Dyar (1904:40–41) from British Columbia as this species. Ludlow (1905:231–2) described *Taeniorhynchus sierrensis* from several males and females sent from Three Rivers (Tulare County), California and subsequently (Ludlow, 1906:132) synonymized it with *varipalpus*. Since that time the common tree-hole *Aedes* of Western North America has been identified as "*varipalpus*" and has now been reported in literature from British Columbia, Washington, Oregon, California, Nevada, Arizona and Baja California (Carpenter and La Casse, 1955:258; Ross, 1943:86).

With the discovery of *monticola*, it becomes evident that the "*varipalpus*" of authors is actually a complex of two or more sibling species and possibly several subspecies. Following the study of the material from Southern Arizona and its comparison with material from California, it appeared that we might be dealing with two species, one restricted to the Pacific Slope and characterized in the adults by the absence of a postcoxal scale patch and the presence of a white ring at the base of the fourth hind tarsal segment, and the other restricted to the mountains in the interior and characterized by the presence of the scale patch and the absence of the white ring. However, the situation is much more complex. First, the specimens from Baja California possess both diagnostic features of the Southern Arizona form, although they differ in other details. Second, and much more significant, the holotype female of *varipalpus* examined for us by Dr. Stone, lacks both the postcoxal scale patch and the white ring. We have come to the conclusion that *monticola* is a distinct species characterized by the presence of the postcoxal scale patch and that it is represented in Baja California by probably a distinct subspecies. We suspect that the Pacific Slope populations, including the Nevada record from Glenbrook (adjacent to Lake Tahoe), constitute a species distinct from the true *varipalpus* from Central Arizona mountains, and further that they may break up into two or more subspecies, as we find Southern California males exhibiting constant differences from all other California populations we have seen. Solution of this problem must await study of topotypic males, larvae, pupae and additional females, and a comparison of material from the entire range of "*varipalpus*." It appears to us that this complex is prone to speciation and that additional distinct populations exist in unsuspected areas in Western North America. Dr. D. W. Jenkins (1956, *in lit.*) informs us that he has reared a large number of adults of "*varipalpus*" from larvae collected in cottonwood tree-holes near Hamilton, Montana. This material has not been studied as yet. Should our surmises prove correct, the name *sierrensis* may have to be resurrected for at least some of the populations of the Pacific Slope. For the present, we are using "*varipalpus*" for all the populations of the complex other than *monticola* and the true *varipalpus* from Williams, Ariz.

As indicated in the diagnosis, *monticola* is extremely similar to "*varipalpus*", Auct. (as described and figured in Carpenter & La Casse, 1955:257–9) in most characters of the adults, larvae and pupae and is distinguished from it in these stages by relatively few characters. Since the two forms are tree-hole breeders and are allopatric, it might be considered that they are merely subspecifically distinct. The striking difference in the male genitalia as well as the constancy of the differential characters, particularly the presence of the postcoxal scale patch of the adults, the shape of the pupal paddle, and the absence of hair 11 on abdominal segment I of the larva, lead us to the interpretation that *monticola* is a distinct species. We consider that the similarities with "*varipalpus*" are genetic despite the unexpected male genitalic differences and are not due to convergence developed concomitant to the utilization of similar breeding habitats.

No attempt has been made by American workers in the past to determine the affinities of *Aedes varipalpus*. It has stood as an anomalous element in the mosquito fauna of North America since its description and has been shuffled back and forth among the various subgenera of *Aedes*. Following Edwards (1932:154), *varipalpus* has been placed in *Finlaya*, chiefly on the basis of breeding habitat and some male characters. Knight and Marks (1952:517) removed it from *Finlaya* because of differences in female genitalia but did not assign it to any other subgenus. A search for Old World forms related to *monticola* reveals that the *varipalpus* complex has many similarities, as indicated by Martini (1930:319), with the *Aedes pulchritarsis* complex which occurs in Europe, the Mediterranean area, South Russia and extends into Central Asia, India (Barraud, 1934:150–2) and even into northern Thailand (Thurman, 1956:222). All members of the two complexes, so far as is known, are tree-hole breeders and have a similar type of ornamentation. The male genitalia of *monticola* appear to be very close to those of *A. pulchritarsis* (Rondani, 1872) as figured by Martini (1930: 318, fig. 358) and its larva shows some similarities

with that of *A. stegomyina* Stackelberg & Montchadsky, 1926 (Montchadsky, 1926:153, fig. 2). We are convinced that the *varipalpus* complex is the New World offshoot of the *pulchritarsis* complex. On the basis of the simplicity of the male genitalia we may speculate even further that *monticola* represents the relict remnants of the parental stock of the *varipalpus* complex from which the more specialized modern "*varipalpus*" has evolved but, at present, we cannot even guess the relation of the true *varipalpus* to the other forms.

Edwards (1932:141) placed *pulchritarsis* in group E (*dorsalis*) of *Ochlerotatus*, apparently chiefly on the basis of superficial similarity in tarsal ornamentation. We believe that *pulchritarsis*, *varipalpus* and related forms should be recognized as a separate group with a tentative characterization as given by Edwards (1932:141) for the second of the two subgroups he recognized. This *pulchritarsis-varipalpus* group appears to be an ancient one, with a number of isolated relict populations scattered in the Old as well as the New World. Its typical members are tree-hole breeders but it is probable that *A. mariae* (Ed. & Et. Sergent, 1903), which breeds in rock holes, also belongs in the group. There are a number of superficial similarities between this group and some *Finlaya*. It appears to us that these similarities are due to convergence but it is quite possible that the group is close to the stem from which *Finlaya* originated. For the present, we prefer to retain the group in *Ochlerotatus*, pending a much needed revision of this complex subgenus and its various derivatives.

In addition to the *varipalpus* complex, two species of tree-hole breeding *Aedes* have been reported from Western North America, namely *A. muelleri* Dyar, 1920 and *A. purpureipes* Aitken, 1941 (McDonald, 1956a, b). The affinities of both species have been obscure. We find that the male genitalia of these two species are very similar to those of *monticola* (apical lobe undeveloped; basal lobe small or indistinct and with a single differentiated seta) and further that their larvae and pupae share a number of characters with *monticola* (particularly position of hairs 1 and 2 on abdominal segments IV and V of the pupa and absence of hair 11 on segment I of the larva) and differ in these respects from other North American *Aedes*. Although it is quite possible that some of the similarities may be due to convergence, they are so numerous that we believe they are indicative of rather close relationship. *Aedes muelleri* departs from the *varipalpus* complex chiefly in ornamentation of the adults (different mesonotal pattern, entirely dark tarsi) and in the absence of teeth on the fore and mid claws of the female (but not of the male). We would place *muelleri* as an aberrant member of the *pulchritarsis-varipalpus* group despite the toothless claws, for although this character has not been reported, to our knowledge, in any North American *Ochlerotatus*, it is known in some races of Palaerctic species assigned to this subgenus. On the other hand *purpureipes*, although it has many similarities with this group, is strikingly different in some characters of the larva (spiculose integument, ventral brush, anal gills, etc. . . .) and particularly in the absence of postspiracular bristles in the adults, which also have an entirely different type of ornamentation. It appears to us that this species is probably a derivative of the *pulchritarsis-varipalpus* group. However, its distinctive characters are so unique, at present, among New World *Aedes* that we prefer to leave it in the monotypic subgenus *Kompia*.

We have made no attempt to identify the *pulchritarsis-varipalpus* group in the mosquito fauna of Central and South America but it would seem likely that it is represented in these regions, particularly in montane areas, and that at least some of the tree-hole breeding *Ochlerotatus* reported from the Neotropical region belong to it. Before any changes are made in the subgeneric assignment of this group and its derivatives it will be necessary to examine carefully all such forms.

ADDITIONS TO EXISTING KEYS

To facilitate the recognition of *Aedes monticola* and other known tree-hole breeding *Aedes* of Western North America we present the following modifications and additions to the keys of Carpenter and La Casse (1955), using their terminology and style.

ADULT FEMALES—p. 139

24(23). Palpi entirely dark; scutellum with narrow yellowish scales . . . . **atropalpus** (Coq.), p. 253
Palpi with white bands; scutellum with broad white scales 24a

24a(24). Postcoxal scale tuft present . . . . **monticola** Belk. & McD.
Postcoxal scale tuft absent 24b

24b(24a). Fourth hind tarsal segment dark at base . . . . **varipalpus** (Coq.)
Fourth hind tarsal segment with a distinct basal white ring . . . . **"varipalpus"**, Auct., p. 257

Note: Females of *purpureipes* and *muelleri* run satisfactorily in the existing keys to couplets 26 and 54 respectively.

MALE TERMINALIA—p. 141

9(8). Basal lobe of basistyle with one or more strong differentiated setae 9a
Basal lobe of basistyle without strong differentiated setae but with numerous slender hairs 10

9a(9). Basal lobe of basistyle with a dense clump of long strong apically curved setae . . . . **"varipalpus"**, Auct., p. 257
Basal lobe of basistyle with only one strong seta differentiated 9b

9b(9a). Differentiated seta of basal lobe nearly straight, not sharply curved nor hooked on apex; basal lobe hardly differentiated but with extensive area covered with numerous short, weak hairs . . . . **purpureipes** Aitken, p. 220

Differentiated seta of basal lobe sharply curved or hooked on apex; basal lobe moderately prominent and with a smaller area covered with few hairs of different sizes.....9c

9c(9b). Differentiated seta of basal lobe strongly attenuated and sharply curved on apex; claspette filament sharply attenuated and abruptly curved on apex; lobes of ninth tergite each with seven or more strong setae.....**monticola** Belk. & McD.

Differentiated seta of basal lobe not strongly attenuated but abruptly hooked on apex; claspette filament gradually attenuated and without abrupt curvature on apex; lobes of ninth tergite each with two to four strong setae.....**muelleri** Dyar

Note: Male terminalia of *varipalpus* (Coq.) undescribed.

LARVAE (FOURTH INSTAR)—p. 146

40(39). Integument of thorax and abdomen strongly spiculate.....**purpureipes** Aitken, p. 220

Integument of thorax and abdomen smooth.....40a

40a(40). Long hairs of ventral brush of anal segment multiple, usually with four branches; anal gills markedly unequal, ventral pair smaller, dorsal pair rarely more than twice as long as the saddle of the anal segment.....41

Long hairs of ventral brush usually single or double; anal gills subequal, at least two and one-half times as long as the saddle.....41a

41a(40a). Individual comb scale elongated into an unfringed spine; most caudal hair of ventral brush (4a) a short multiple tuft.....**muelleri** Dyar

Individual comb scale broadly rounded and fringed on apex; most caudal hair of ventral brush long and single.....41b

41b(41a). Hair 11 of first abdominal segment present.....**"varipalpus"**, Auct., p. 258

Hair 11 of first abdominal segment absent.....**monticola** Belk. & McD.

Note: Larva of *varipalpus* (Coq.) undescribed.

ADDENDUM

In August 1956 we were fortunate in obtaining all stages and in making individual rearings of 20 males and 22 females of *A. varipalpus* from the type locality of Williams, Arizona. Although this material has not been studied in detail as yet and will be fully described at a later date, we should like to indicate at this time the outstanding characteristics of *varipalpus* so that the necessary corrections can be made in the text and keys of this paper. As we surmised, *varipalpus* Coq. is a species distinct from both *monticola* and the "*varipalpus*" of the Pacific Slope, which now should be called *Aedes* (*O.*) *sierrensis* (Ludlow, 1905). In most respects *varipalpus* is closer to *monticola* than to *sierrensis*. The male genitalia of the first two species are similar and conspicuously different from those of *sierrensis* in the presence of only one differentiated bristle instead of a clump of many stout bristles on the basal lobe of the sidepiece. *A. varipalpus* differs from *monticola*, however, in the presence of numerous long slender bristles, instead of 1 or 2, ventrad of the differentiated bristle of the basal lobe and in addition has a differently shaped claspette filament. Adults of both sexes of *varipalpus* can be told from the other two known species by the presence of several light bristles among the scales of the subspiracular patch, a very unusual character not reported previously for any mosquito to our knowledge. The coloration of the fourth hind tarsal segment of *varipalpus* is variable, some specimens showing a very narrow basal pale ring, others a slight dorsal pale patch but never a broad basal pale ring as in *sierrensis*. The larva of *varipalpus* is similar to that of *monticola* in lacking hair 11–I but differs from it in details of chaetotaxy, shape of siphon and length of anal gills. The pupal stage has not been studied as yet.

It is of interest to note that we also found *varipalpus* in the Hualpai Mts., an isolated range near Kingman, Ariz., some 100 airline miles west of Williams. In both areas *varipalpus* larvae were scarce and were associated with those of *Aedes muelleri*, the latter species being dominant at Williams.

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AN EXPLORATORY INVESTIGATION OF THE EFFECT IN VIVO AND IN VITRO OF COCKROACH TISSUES ON THE VIABILITY OF NEWCASTLE DISEASE VIRUS[1]

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Some microorganisms which are pathogenic to higher animals exhibit pathogenicity to insects also, whereas a number of non-pathogenic organisms are quite virulent for insects. However, as a group, insects in general have a relatively high natural immunity to most organisms which are pathogenic to higher animals. Moreover, the rate of immunization is extremely rapid for insects, usually being accomplished within 24 hours after injection of the antigen. For these reasons the prospects of employing insects in virus studies appear to offer great promise. Although considerable research has been conducted on the polyhedral diseases which affect many insects, and on the rickettsial and viral diseases of which insects are the known or suspected vectors, there has been very little investigation of the effects of insects on the viability of foreign viruses.

As early as 1922, Macfie observed that cockroaches could ingest pathogenic bacteria and subsequently excrete these in a viable condition, thus suggesting the possibility of the role of roaches as disease carriers. Wedberg and Clarke (1947) devised a method for the quantitative measurement of the possible role of insects in the transmission of diseases through infected excreta. This appears to have given impetus to research along such lines; for shortly after, it was shown (Mackerras and Mackerras, 1948; Mackerras and Pope, 1948; Olson and Rueger, 1950) that the cockroach was able to excrete viable *Salmonella* for a period of at least 5 days following ingestion of the infectious agent.

Syverton and Fischer (1950) demonstrated that the cockroach is capable of harboring intracorporeally for at least 7 days the virus of spontaneous mouse encephalomyelitis and that during this period it excreted daily dosages sufficient to kill test mice. Hurlbut (1950) injected poliomyelitis virus into the haemocoele of the American roach and found that the virus remained viable for at least 15 days. Fischer and Syverton (1951) showed that roaches fed a single dose of Coxsackie virus were capable of excreting daily over a period of as many as 15 days sufficient virus to kill test mice.

It is possible that the passage of certain viruses through insects will alter the properties of the virus, and if so, such technique would have important widespread application. On the one hand insects may prove to be satisfactory culture media for virus, or they may have an inhibitory effect on the growth of certain disease agents. In view of the dearth of information concerning this it appears extremely worthwhile to make preliminary studies. Newcastle was selected as the agent because of its ease of handling, and facile characterization. The American cockroach (*Periplaneta americana* L.) was chosen as the subject for reasons of availability, size, and available information on physiology and biochemistry of the species.

MATERIALS AND METHODS

Virus Strain Used. The virus employed in these tests was the California strain of Newcastle, No. NC 194–5–6–7, and unless otherwise specified was obtained January 7, 1952. This culture was filtered through a Berkefeld January 12, 1952.

Inoculation of Chicken Embryos. Unless otherwise stated all inoculations were made into the allantoic cavity of 9- to 10 day-old developing chicken embryos (white leghorn) according to the procedure described by Beaudette *et al.* (1952). Two-tenths cc. of inoculum was employed in each instance.

Injection of Roaches with Virus. Both sexes of the adult (6 to 7 months old) American cockroach were employed in these studies. Each insect received 0.05 cc. of the virus preparation by means of a micro-injection apparatus which has been previously described. To facilitate handling, the insects were anesthetized with CO_2 prior to injection. The treated insects were then placed in containers which were specially designed for the facile collection of feces (described further on).

Feeding Virus to Roaches. Individuals of both sexes were fed 0.03 or 0.05 cc. of the virus preparation using the same micro-injection apparatus employed in the injection studies. In this case, however, the insects were not anesthetized, but were held in a relatively quiescent position while feeding.

Collection of Feces. To facilitate the collection

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of feces the insects were confined in glass cylinders 8 inches high and 3 inches in diameter. The upper 2 inches of the cylinder were coated with vaseline on the inside and covered with a piece of surgical gauze to prevent escape of the insects. The cylinder was placed on a piece of screen with ¼ inch openings which in turn rested on an open petri dish. The fecal pellets, which usually dropped unimpeded into the petri dish, were collected at regular intervals. Food and water were placed in small beakers, which were taped to the sides of the cylinders above the wire base to prevent interference with the pellet collection.

Preparation of Feces for Inoculation. Samples of fecal pellets were ground in a mortar and suspended in 1 cc. of broth. The mixture was then centrifuged for 10 minutes in a clinical centrifuge at 1500 r.p.m., 10,000 units of penicillin and 10 mgs. of streptomycin were added to the supernatant, and the preparation was incubated for 15 minutes at 37°C. The preparation was then placed in the freezer and the inoculations were performed on the following day.

Preparation of Virus-Inoculated Roaches for Inoculation of Chick Embryos. Two individuals of each sex were randomly selected at definite time intervals following inoculation with virus. Each group (two males or two females) were frozen, ground with sterile sand, and suspended in 4 cc. of broth. The mixture was centrifuged for 15 minutes, and the aqueous phase was removed and treated with 10,000 units each of penicillin and 10 mgs. of streptomycin. Following a 15 minute incubation period, the preparation was inoculated into chick embryos.

Preparation of Virus-Fed Roaches for Inoculation of Chick Embryos. Two individuals of each sex were randomly selected at definite time intervals after feeding with virus. These were treated in the same manner as that described for the virus-inoculated insects.

In Vitro Studies on the Intestinal Tract. The alimentary canal was removed from five roaches of each sex and divided into two groups (male and female). To prevent loss of intestinal contents the tract was tied with thread at both ends prior to severance from the body. Each group of alimentary canals was ground with sterile sand in a mortar and suspended in broth. Following a 5 minute centrifugation period the supernatant was treated with antibiotics in the usual manner and incubated at 37°C. for 15 minutes. This extract was mixed with virus in different proportions and inoculated into chick embryos. Ten-fold virus dilutions were utilized.

To one volume of the different dilutions there was added one volume of the roach tissue extract. As a control broth was substituted in equal volumes for the extract.

In Vitro Studies on the Effect of Intestinal Bacteria on Virus. Extracts of feces and digestive tracts were prepared in the usual manner and cultured on agar. The colonies obtained in this manner were cultured in broth and incubated at 37°C. for 24 hours, and placed in the refrigerator. Six days later the cultures were centrifuged and the supernatant treated with antibiotics in the usual manner. This preparation was added to different dilutions of virus and incubated at room temperature for 15 minutes prior to inoculation. Virus controls were prepared in the usual manner.

In Vitro Studies on Extracts of Entire Roaches. Only males were employed in this study. The roaches were frozen, ground with sterile sand, suspended in broth, and centrifuged for 15 minutes. One volume of the supernatant was added to one volume of each of several virus dilutions as previously described and these were incubated at 37°C. for 15 minutes.

EXPERIMENTAL

1. Attempts to recover virus from feces of cockroaches infected by feeding

Male and female roaches were fed 0.03 cc. of the virus preparation. Suspensions were made of

TABLE I

THE *in vivo* EFFECT OF MALE AND FEMALE ROACHES ON THE VIABILITY OF INGESTED NEWCASTLE VIRUS

SEX	INTERVAL BETWEEN FEEDING AND FREEZING (HRS.)	NO. OF EGGS INOCULATED	MORTALITY (HOURS)					
			24	48	72	96	120	144
Male	2	4	—	4	—	—	—	—
"	18	4	1	—	3	—	—	—
"	51	4	1	—	1	—	—	—
"	68	4	1	—	—	—	—	—
"	94	4	—	—	—	—	—	—
"	118	4	2	—	—	—	—	—
Female	2	4	—	—	4	—	—	—
"	28	4	—	1	3	—	—	—
"	52	4	1	—	—	—	—	—
"	76	4	—	—	—	—	—	—
"	100	4	1	—	—	—	—	—
"	124	4	1	—	—	—	—	—

fecal pellets collected at intervals of 4 hours, 1, 1½, 2 days and each succeeding 24 hours for a total period of 38 days. Each suspension was inoculated in a dose of 0.2 cc. per egg to test for the presence of virus. The 4-hour sample was inoculated into three eggs but each of the others was inoculated into four eggs. The eggs were incubated 7 days post-inoculation and dead embryos were removed on daily candling. Embryos dead within 24 hours were discarded as is customary with this virus. In no case did a suspension kill all embryos to give a typical picture of the action of Newcastle disease virus. A total of 8 embryos in the male roach series and 11 in the female series died, exclusive of 24-hour dead, and a liver suspension of each dead embryo was sub-inoculated to test for virus. In no case was live virus recovered.

The roaches employed in the feeding tests were frozen 35½ days following feeding with the virus. Roaches which received no virus were used as checks. To determine if the virus remained in the insect, extracts of the ground insects were prepared and inoculated into developing chicken embryos. Six eggs were used for each test group. Candling was done daily for 6 days and in no case was the virus recovered.

Although the virus was not recovered from the feces at any period during the 38-day interval following feeding and was not present in the insects at the end of the 38 days, the possibility existed that the virus was retained within the insect in a viable condition for a portion of the 38 days.

To explore this possibility the following experiments were conducted.

2. Attempts to recover virus from cockroaches infected by feeding

Male and female roaches were fed 0.05 cc. of the virus preparation. At definite periods after feeding several of the insects were frozen and extracts of the tissues were prepared. Developing chick embryos were inoculated with 0.2 cc. of these preparations and incubated as usual for 7 days. The results are shown in Table I.

TABLE II

Effect of antibiotic-Treated Broth Cultures of Roach Fecal Bacteria on Newcastle Disease Virus

Culture No.	No. of Eggs Inoculated	Virus Dilution	Mortality (Hours)				
			24	48	72	96	120
1	4	10^{-8}	1	–	3	–	—
1	4	10^{-9}	1	–	2	1	—
2	4	10^{-8}	3	–	1	–	—
2	4	10^{-9}	2	–	2	–	—
3	4	10^{-8}	1	–	3	–	—
3	3	10^{-9}	–	–	3	–	—
Control	5	10^{-8}	2	–	3	–	—
Control	5	10^{-9}	–	–	3	1	—
Control	5	10^{-10}	1	–	2	–	—

It is possible to recover the virus up to 28 hours after infection in the case of female roaches. Virus was recovered at 18 hours from the males and it is probable that recovery would have been possible at 28 hours. Between 18 to 51 hours (in the males) and 28 to 52 hours (in the females) after ingestion of the virus, the viability of the virus is greatly reduced. The fact that the virus could not be recovered after 28 hours suggests the presence of antiviral activity in the roach, possibly in the alimentary canal.

Since the flora of the digestive tract of the roach is quite extensive, it was desirable next to consider the possibility of antiviral activity on the part of these organisms. To explore this possibility the following experiments were performed.

3. Effect of antibiotic-treated broth cultures of bacteria isolated from the alimentary canal

TABLE III

The Effect of Bacteria Isolated from the Alimentary Canal of the Roach on Newcastle Disease Virus

Culture No.	Number of Inoculations	Virus Dilution	Sex of Roach	Mortality (hours)				
				24	48	72	96	120
1	4	10^{-8}	Female	—	—	4	—	—
1	4	10^{-9}	Female	—	—	4	—	—
2	4	10^{-8}	Female	—	—	4	—	—
2	4	10^{-9}	Female	2	—	—	1	—
3	4	10^{-8}	Female	—	—	4	—	—
3	4	10^{-9}	Female	—	—	3	1	—
1	4	10^{-8}	Male	1	—	3	—	—
1	4	10^{-9}	Male	2	—	1	1	—
2	4	10^{-8}	Male	—	—	4	—	—
2	4	10^{-9}	Male	—	—	2	—	—
Control	5	10^{-8}	———	2	—	3	—	—
Control	5	10^{-9}	———	—	—	3	1	—
Control	5	10^{-10}	———	1	—	2	—	—

TABLE IV

THE EFFECT OF EXTRACTS OF THE ALIMENTARY CANAL OF ROACHES ON THE VIABILITY OF NEWCASTLE DISEASE VIRUS

SEX OF ROACH	NO. OF EGGS INOCULATED	VIRUS DILUTION	MORTALITY (HOURS)					
			24	48	72	96	120	144
Males	5	10^{-4}	—	—	1	3	1	—
Males	5	10^{-5}	2	—	—	2	1	—
Males	5	10^{-6}	1	—	—	4	—	—
Males	5	10^{-7}	—	—	—	4	1	—
Females	5	10^{-4}	—	—	2	2	1	—
Females	5	10^{-5}	2	—	2	1	—	—
Females	5	10^{-6}	2	—	—	—	—	—
Females	5	10^{-7}	1	—	—	1	—	—
Control	5	10^{-4}	2	—	1	2	—	—
Control	5	10^{-5}	1	—	—	4	—	—
Control	5	10^{-6}	—	—	—	5	—	—
Control	6	10^{-7}	1	—	1	4	—	—

and from fecal matter of roaches on Newcastle disease virus

A. *Bacteria isolated from feces.* Extracts of fecal material were prepared in the usual manner and cultured on agar plates. There were at least three different types of colonies, none of which fermented dextrose, lactose, maltose, or sucrose. Broth cultures of the three types were treated with antibiotics as previously described and equal amounts incubated with 10^{-8} and 10^{-9} dilutions of a virus suspension that titered 10^{-9}.

B. *Bacteria isolated from alimentary canal.* Several types of bacteria were isolated from the extracts of the digestive tracts of male and female roaches. The cultures obtained from the females fermented all sugars except lactose without the formation of gas. One type of bacteria from the male extract fermented dextrose and maltose. A second type fermented dextrose and maltose with a production of gas. Eggs were inoculated as previously described with a mixture of these antibiotic-treated cultures and virus.

Apparently antibiotic-treated broth cultures of intestinal flora are incapable of inactivating Newcastle disease virus (Tables II, III). This then means that if an antiviral factor truly exists in the digestive tract of the roach, the activity must reside in or result from the tissues *per se.*

4. Effect of extracts of fecal material and extracts of alimentary canal on Newcastle disease virus

Extracts of fecal material and extracts of ground alimentary canals of male and female roaches were prepared and tested in the usual manner. The fecal preparations had no effect on the viability of the virus. The results of the tests on the tissue extracts are shown in Tables IV and V.

There appears to be a slight activity on the part of the tissues of the digestive tract, although it has not lent itself too well to reproducibility.

Seven-day old eggs were inoculated on the chorioallantoic membrane with 0.2 cc. of an extract of the alimentary canals of roaches.

TABLE V

THE EFFECTS OF EXTRACTS OF THE ALIMENTARY CANAL OF ROACHES ON THE VIABILITY OF NEWCASTLE DISEASE VIRUS

SEX OF ROACH	NO. OF EGGS INOCULATED	VIRUS DILUTION	MORTALITY (HOURS)					
			24	48	72	96	120	144
Male	4	10^{-6}	—	3	1	—	—	—
Male	4	10^{-7}	—	1	3	—	—	—
Male	4	10^{-8}	1	—	—	1	—	—
Male	4	10^{-9}	—	2	—	—	—	—
Female	4	10^{-5}	—	4	—	—	—	—
Female	4	10^{-6}	1	1	2	—	—	—
Female	4	10^{-7}	—	3	—	—	—	—
Female	4	10^{-8}	2	1	—	—	—	—
Control	4	10^{-5}	—	4	—	—	—	—
Control	4	10^{-6}	1	3	—	—	—	—
Control	4	10^{-7}	—	3	1	—	—	—
Control	4	10^{-8}	—	—	4	—	—	—
Control	4	10^{-9}	—	—	3	—	—	—

TABLE VI

THE *in vivo* EFFECT OF MALE AND FEMALE ROACHES ON THE VIABILITY OF NEWCASTLE DISEASE VIRUS WHEN INJECTED

SEX	NO. OF EGGS INOCULATED	INTERVAL BETWEEN INJECTION AND FREEZING. DAYS	MORTALITY (HOURS)					
			24	48	72	96	120	144
Male	4	Immediately after inject.	—	4	—	—	—	—
"	4	1 day	1	3	—	—	—	—
"	4	2 days	1	2	1	—	—	—
"	4	3 "	—	2	2	—	—	—
"	4	4 "	1	—	1	2	—	—
"	4	5 "	1	3	—	—	—	—
Female	4	Immediately after inject.	1	3	—	—	—	—
"	4	1 day	—	4	—	—	—	—
"	4	2 days	1	3	—	—	—	—
"	4	3 "	1	2	1	—	—	—
"	4	4 "	—	2	2	—	—	—
"	4	5 "	—	4	—	—	—	—

Three days later the eggs were inoculated at the same point with the virus. Subsequent tests showed that the preparation had no effect on the titer of the virus.

Before proceeding further it was deemed advisable to conduct injection studies similar to those used in the feeding experiments.

5. Attempts to recover virus from feces of cockroaches infected by injection

Male and female roaches were injected with 0.05 cc. of virus. Suspensions were made of fecal pellets collected daily for the first 7 days and at 3 day intervals for the next 28 days. For each fecal sample four eggs were inoculated with 0.2 cc. of the suspension to test for the presence of virus. The eggs were inspected daily as previously described. There was no evidence of virus recovery in any of the samples.

The roaches employed in the injection experiments were frozen 35½ days following treatment with virus. To determine if the virus remained in the insect, extracts of the ground insects were prepared and inoculated into developing chicken embryos. A preparation of uninfected roaches was utilized for the control. Six eggs were used for each sample and these were treated in the usual manner. Newcastle disease virus was not detected in any of the preparations.

Since the virus when injected into the bloodstream of the roach is not eliminated in detectable amounts in the feces and at 36 days after infection it could not be recovered from the insects, it appears probable that it is being destroyed within the body.

6. Attempts to recover virus from cockroaches infected by injection

Male and female roaches were injected with virus, and extracts of the tissues were made at definite time intervals thereafter according to the procedure previously described. Developing chicken embryos were inoculated with 0.2 cc. of the extract. The results are shown in Table VI.

The viability of virus is apparently not appreciably reduced after 5 days in the bloodstream of the roach. To determine any reduction in the concentration of virus, extracts of female cockroaches inoculated 24 and 96 hours previously were titered.

TABLE VII

TITRATION OF NEWCASTLE DISEASE VIRUS IN ROACH TISSUES PREPARED 24 HOURS AFTER INJECTION

NO. OF EGGS INOCULATED	EXTRACT DILUTION	MORTALITY (HOURS)					
		24	48	72	96	120	144
4	10^{-2}	—	2	2	—	—	—
4	10^{-3}	1	—	—	1	1	—
4	10^{-4}	—	—	—	—	—	—
4	10^{-5}	—	—	—	—	—	—
4	10^{-6}	—	—	—	—	—	—

The results in Tables VII and VIII show that the titer of the virus is significantly reduced 4 days after introduction into the bloodstream.

If there are present in the roach substances which have the capacity for inactivation of Newcastle disease virus, these substances do not function in extracts of the tissues; for preparations of extracts of entire roaches were incapable of reducing the titer when mixed with a series of virus dilutions.

DISCUSSION

The failure to recover Newcastle disease virus from feces when fed to cockroaches suggests the possibility of the presence of an anti-viral factor in the alimentary canal. Normally, ingested substances will begin to appear in the feces of the roach approximately 9 hours after feeding (Snipes and Tauber 1937). Unless a selective retention has occurred, and this seems unlikely, we must consider that the viability has been impaired during the process of passing thru the

digestive tract. As shown by assays of intestines of roaches which were fed the virus, the titer is appreciably reduced at some point between 28 and 51 hours after feeding (Table I). It has been demonstrated that the digestive tracts of certain insects are capable of exerting bactericidal action. In some cases the effect is one of pH whereas in others it is considered due primarily to the action of bacteria which are normally present

TABLE VIII

TITRATION OF NEWCASTLE DISEASE VIRUS OF ROACH TISSUES PREPARED 4 DAYS AFTER INJECTION

No. of Inoculations	Extract Dilution	Mortality (Hours)				
		24	48	96	120	144
5	10^{-2}	—	1	—	—	—
4	10^{-3}	—	—	—	—	—
4	10^{-4}	—	—	—	—	—
4	10^{-5}	1	—	—	—	—

in the alimentary canal. The destruction of Newcastle disease virus demonstrated in this study can not be explained by either factor; for cultures of the intestinal flora were incapable of reducing the titer of the virus and the pH (saliva 6.9, Wigglesworth 1927; crop 5.2; stomach 6.2; intestine 7.1; and rectum 7.6, Grayson 1951) of the tract is within the range which the virus will tolerate. Rendtorff and Francis (1943) found that the Lansing mouse—adapted strain of human poliomyelitis virus when fed to house flies, *Musca domestica* L., persisted primarily in the abdomen but exhibited a greatly decreased titer after 7 hours. They believed that the loss was mainly the result of excretion since a considerable amount of active virus was recovered from fecal and vomit spots. This did not appear to be the case in the present studies since no virus was detected in the fecal pellets. That this phenomenon occurs within the alimentary canal is evidenced by the fact that roaches injected with the virus remain virulent for 5 days, and possibly longer, after treatment, although the titer decreases significantly between the first and fourth days following injection. The effect does not appear to be one of simple denaturation since the pH of the digestive tract is within the tolerable range, and the destruction time is considerably less than that necessary for breakdown on standing at room temperature. There are proteolytic enzymes present in the alimentary canal, and it is possible that the virus has been treated as any food substance and is the victim of digestive processes. The flora of the intestine do not appear to be concerned in the inactivation, at least according to the known principles of antibiotic action; for incubation with cultures of intestinal bacteria had no effect on the viability of the virus. Similarly, in vitro tests with extracts of roach tissues failed to demonstrate antiviral activity.

Inasmuch as this is an exploratory investigation, there is no satisfactory basis for postulation, and the only conclusion that may be validly drawn is that for some obscure reason Newcastle disease virus could not be recovered after feeding to roaches.

SUMMARY

1. Techniques are described for the treatment of roaches and other large insects with viral preparations.

2. Newcastle disease virus fed to roaches could not be recovered in the fecal material.

3. The viability of Newcastle disease virus was greatly reduced during the interval of 28 to 51 hours after feeding to roaches.

4. The viability of Newcastle disease virus was much less strongly affected when injected into the bloodstream of roaches.

5. Incubation with extracts of entire roaches, digestive tracts, fecal material, and cultures of intestinal bacteria had no effect on the viability of Newcastle disease virus.

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NOMENCLATURAL CHANGES AND TYPE DESIGNATIONS OF SOME NEW WORLD AGROMYZIDAE (DIPTERA)[1,2]

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A study of the types of all but one of the agromyzid species that are deposited in United States museums has resulted in several new synonymies, new generic positions, the resurrection of a number of species, and other nomenclatural changes. All such changes are published at this time so that the correct binomina may be used in a forthcoming synopsis of the Nearctic Agromyzidae. It is planned that the synopsis will give keys and characters with which to separate difficult complexes, figures and diagnostic characters of many of the types and typical specimens of Holarctic species, as well as distributional and larval host plant data.

A number of descriptions have been based upon syntypes. In accordance with the Copenhagen Decisions on Zoological Nomenclature (Heming, 1953, par. 136, pg. 72), lectotypes are herein designated for all such species so that each may be defined objectively rather than subjectively. Also, in certain instances, careful selection of a lectotype was needed in order to preserve a name of relatively long standing. In no case is the designation of a neotype anticipated since none of the species for which the holotype cannot be found presents a problem of stability or a confused zoological problem (Heming, 1953, par. 34, pg. 28).

LECTOTYPE DESIGNATIONS

Frison (1927) designated types for all of the species at the Illinois Natural History Survey. For each of Malloch's holotypes he used the specimen bearing Malloch's handwritten label. Allotypes, except for a few species, were not labelled by Malloch, so Frison chose a specimen of the sex opposite that of the type, labelling the rest of the series as paratypes.

Loew's species frequently were based upon two or more specimens. For each species, except *Phytomyza ilicicola*, I have designated the specimen bearing a label with the specific name followed by "m" (="mihi"). The labelled specimen of *P. ilicicola* has the head missing, so I chose a specimen from the type series of its sex.

Dr. Melander has told me on two occasions that for his species the first specimen in each series is to be considered the holotype, but in the absence of a definite published designation that need not be binding. Not infrequently such specimens were in the poorest condition or of a species different from that of the majority of the specimens and as described in his paper (1913). Therefore, for each lectotype I chose the best of its series or, for *Agromyza lima* and *A. nasuta*, the best specimen of the sex described by Melander. Each lectotype is marked with a "T" on the underside of its label and placed first in its respective series.

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SYSTEMATIC TREATMENT

Genus **Agromyza** Fallén

Agromyza anthrax Williston, *nomen dubium*

Agromyza anthrax Williston, 1896, Trans. Ent. Soc. London, 1896: 430.

The single specimen upon which this species was based, a male from St. Vincent, West Indies,

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is neither in the British Museum nor in the Snow Entomological Museum. Since the description is inadequate to characterize the species and since the type is apparently lost, *anthrax* is considered a *nomen dubium*.

Agromyza aristata Malloch

Agromyza aristata Malloch, 1915, Canad. Ent., 47: 13.
Phytagromyza aristata (Malloch): Frick, 1952, Univ. Calif. Publ. Ent., 8: 416.
Agromyza ulmi Frost, 1924, Cornell Univ. Agric. Exp. Sta. Mem., 78: 54; Shewell, 1953, Canad. Ent., 85: 469. New synonymy.

Holotype ♀ (I.N.H.S.).—Havana, Illinois, Gleason's sand dune, IV–30–1914 (C. A. Hart and J. R. Malloch). Type of synonym: holotype ♂ (U.S.N.M. Type No. 50,033).—Arendtsville, Pennsylvania, IV–22–1923 (S. W. Frost). This species belongs in *Agromyza* and to the so-called *sulphuriceps* (now *rubi*) group (Hendel, 1931). Frost's species is unmistakably the same as *aristata*.

Agromyza inaequalis Malloch

Agromyza inaequalis Malloch, 1914, Proc. Ent. Soc. Washington, 16: 89; Shewell, 1953, Canad. Ent., 85: 463.
Agromyza iridescens Frost, 1936, Ann. Ent. Soc. Amer., 29: 303; Shewell, 1953, Canad. Ent., 85: 463. New synonymy.

Holotype ♀ (U.S.N.M. Type No. 18,344).—Rio Piedras, Puerto Rico, XII–2–1913 (T. H. Jones), P.R.S.G.A. Acc. No. 983–1913. Type of synonym: holotype ♂ (A.M.N.H.).—Barro Colorado Island, Canal Zone, II–16–1929 (C. H. Curran). The type is a female despite Malloch's statement that it is a male. I could find no differences between the types.

Agromyza innominata Williston

Agromyza innominata Williston, 1896, Trans. Ent. Soc. London, 1896: 443; Frick, 1952, Univ. Calif. Publ. Ent., 8: 375.

Holotype ♂ (British Museum).—St. Vincent, West Indies. H. Oldroyd has written (II–28–1956) that this species belongs in the family Milichiidae. Previously Frick had followed Malloch (1913) in excluding the species from the Agromyzidae on the basis of Williston's figure.

Agromyza invaria Walker, 1857, *nomen dubium*

Agromyza invaria Walker, 1857, Trans. Ent. Soc. London, n. ser., 4: 232.

H. Oldroyd has written (I–18–1956) that specimens of this species are not in the British Museum. Walker's brief description is inadequate to diagnose the species and since the type has apparently been lost, *invaria* is considered a *nomen dubium*.

Agromyza nigripes Meigen

Agromyza nigripes Meigen, 1830, Syst. Beschr. Bekann. Eur. Zweifl. Insekt., 6: 170.
Agromyza dubitata Malloch, 1913, Ann. Ent. Soc. Amer., 6: 311; Frick, 1953, Canad. Ent., 85: 69; Shewell, 1953, Canad. Ent., 85: 462. New synonymy.

Type of synonym: holotype ♀ (U.S.N.M. Type No. 15,576).—Beverly, Massachusetts, VII–19–1869 (Burgess). *A. dubitata* is not a synonym of *A. reptans* as stated by Frick (1953). A study of all of the type series showed that it is the same as *nigripes*, a common European species.

Agromyza parvicornis Loew

Agromyza parvicornis Loew, 1869, Berl. Ent. Zeitschr., 13:49; Shewell, 1953, Canad. Ent., 85: 463.
Agromyza neptis (Loew), Coquillett, 1898, U. S. Dept. Agric., Div. Ent. Bull. 10: 78.

Lectotype ♂ (M.C.Z. Type No. 13,441).—Labelled: "Loew coll.; *parvicornis* m." Shewell had the sexes mixed and it is the male that is in good condition. The other specimen, represented by two legs and one wing, is not of the same species because the wing venation is different and not unlike the venation found in species of *Melanagromyza*.

Agromyza rubi Brischke

Agromyza rubi Brischke, 1881, Schrift. Naturf. Ges. Danzig, 5: 250; de Meijere, 1937, Tijdschr. Ent., 80: 170; Hering, 1954, Tijdschr. Ent., 97: 118.
Agromyza sulphuriceps Strobl, 1898, Mitt. Naturwiss. Ver. Steier., 34: 270; Melander, 1913, Jour. N. Y. Ent. Soc., 21: 255; Frick, 1952, Univ. Calif. Publ. Ent., 8: 374.

Hering has recently confirmed this synonymy. All of the specimens under *sulphuriceps* in Melander's collection belong to this species.

Agromyza subnigripes Malloch, 1913

Agromyza subnigripes Malloch, 1913, (*n. n.* for *A. nigripes* Zetterstedt, 1848, *nec* Meigen, 1830), Ann. Ent. Soc. Amer., 6: 334.
Agromyza aprilina Malloch, 1915, Bull. Ill. Lab. Nat. Hist., 11: 359. New synonymy.

Lectotype ♀ (U.S.N.M.).—Hampton, New Hampshire, V–24–1907 (S. A. Shaw), No. 965. Type of synonym: holotype ♀ (I.N.H.S.).—Urbana, Illinois, IV–16–1915 (J. R. Malloch), (? swept from) cottonwood. Malloch described *subnigripes* as having the calypteral fringe black. This probably was the case with the two returned to the Budapest Museum because Hendel (1931) synonymized the species with *mobilis*. However, the fringe is white in the New Hampshire specimen and I am designating this specimen the type of *subnigripes*. *A. aprilina*, a widespread species, is the same as the type of *subnigripes*.

Genus **Melanagromyza** Hendel

Melanagromyza angelicae (Frost)

Agromyza lappae (Loew), *p. p.*, de Meijere, 1924, Tijdschr. Ent., 67: 135; 1925, de Meijere, Tijdschr. Ent., 68: 243; Hendel, 1931, Die Flieg. Palaearkt. Reg., 59: 167.
Agromyza angelicae Frost, 1934, Ent. News, 45: 40.
Melanagromyza angelicae (Frost): Frick, 1952, Univ. Calif. Publ. Ent., 8: 377; Shewell, 1953, Canad. Ent., 85: 463.

Holotype ♂ (U.S.N.M. Type No. 56, 470).—Ithaca, New York, XI–16–1926 (A. S. Mills).

Compared with five European specimens, all reared from *Angelica silvestris*. Recent studies in Europe have shown that this species, mining *Angelica*, is not the same as *lappae*, reared from *Arctium lappa*.

Melanagromyza caerulea (Malloch)

Agromyza caerulea Malloch, 1913, Ann. Ent. Soc. Amer., 6: 322, 334; Bailey and Plank, 1940, Jour. Econ. Ent., 33: 704.
Melanagromyza caerulea (Malloch): Frick, 1952, Univ. Calif. Publ. Ent., 8: 378; Shewell, 1953, Canad. Ent., 85: 464.
Agromyza plumiseta Malloch, 1913, Ann. Ent. Soc. Amer., 6: 324; Frick, 1952, Univ. Calif. Publ. Ent., 8: 378. New synonymy.

Holotype ♀ (U.S.N.M. Type No. 15,584).—Mexico, XI–29–? (S. J. Allende). Type of synonym: holotype ♀ (U.S.N.M. Type No. 15,586).—Bayamon, Puerto Rico, I–1899 (A. Busck). Both types bear a strong spur centrally on the foretibia, although Malloch separated the species by its absence in *plumiseta*. There is considerable variation in the length of the setulae of the arista of the types. These setulae vary in length from one to two times the basal aristal diameter. However, specimens reared from sweet potato and other species of *Ipomoea* show variations as extreme as those of the types. A similar variation in the length of the aristal setulae was noted among 15 specimens of *Melanagromyza aldrichi*.

Melanagromyza minima (Malloch)

Agromyza minima Malloch, 1913, Ann. Ent. Soc. Amer., 6: 328; Frick, 1952, Univ. Calif. Publ. Ent., 8: 382.
Melanagromyza minima (Malloch): Shewell, 1953, Canad. Ent., 85: 468.
Agromyza longicauda Curran, *p. p.*, 1928, Insect Puerto Rico and Virgin Is., 11: 65. New synonymy.
Agromyza longiseta (Malloch), Frost, 1936, Ann. Ent. Soc. Amer., 29: 318.

Holotype ♂ (U.S.N.M. Type No. 15, 590).—Trinidad, West Indies, VI–20–? (A. Busck). Allotype of synonym: allotype ♀ (A.M.N.H.).—St. Croix, Virgin Islands, III–2–1925, F–5,019. The male of *longicauda* belongs in *Ophiomyia lantanae*, while the female with its long ovipositor belongs to *minima*, the females of which have very long ovipositors.

Melanagromyza setifrons (Melander), new combination

Agromyza setifrons Melander, 1913, Jour. N. Y. Ent. Soc., 21: 260.
Ophiomyia setifrons (Melander): Frick, 1952, Univ. Calif. Publ. Ent. 8: 383.

Holotype ♂ (A.L.M.).—Troy, Idaho, VI–14–1908 (W. M. Mann). Frick misplaced the species on the basis of the description.

Melanagromyza simplex (Loew)

Agromyza simplex Loew, 1869, Berl. Ent. Zeitschr., 13: 46.
Melanagromyza simplex (Loew): Hendel, 1920, Arch. Naturgesch., Abt. A, 84: 128; Shewell, 1953, Canad. Ent., 85: 465.

Holotype ♂ (?) (M.C.Z.).—Labelled: "*simplex* m.", and "Gone when I came, Banks." The pin does not bear an M.C.Z. red type label. A neotype is not needed because the name has long been associated with the distinctive miner of asparagus stems.

Melanagromyza tamia (Melander)

Domomyza tamia Melander, 1913, Jour. N. Y. Ent. Soc., 21: 258.
Melanagromyza tamia (Melander): Frick, 1952, Univ. Calif. Publ. Ent., 8: 380.

Lectotype ♀ (A.L.M.).—Wawawai, Washington, V–20–1911 (A. L. Melander). The specimen in the best condition was chosen for the type.

Melanagromyza tiliae (Couden)

Agromyza tiliae Couden, 1908, Proc. Ent. Soc. Washington, 9: 35; Malloch, 1913, Ann. Ent. Soc. Amer., 6: 327.
Melanagromyza tiliae (Couden): Frick, 1952, Univ. Calif. Publ. Ent., 8: 380; Shewell, 1953, Canad. Ent., 85: 465.

Lectotype ♀ (U.S.N.M. Type No. 10,028).—Jennings, Missouri, IV–4–1907 (Mrs. T. F. Hickey), reared from twigs of *Tilia americana*. Each of the adults bears a red label with "TYPE" on it. Couden states that the description was based upon 10 specimens and mentions a cotype specimen. However, he gives the U.S.N.M. number for a single type. There is one specimen, the aforementioned female, in the U.S.N.M. type collection. It has what appears to be the original name label on it. I consider this specimen to be the type.

Melanagromyza virens (Loew)

Agromyza virens Loew, 1869, Berl. Ent. Zeitschr., 13: 46.
Melanagromyza virens (Loew): Frick, 1952, Univ. Calif. Publ. Ent., 8: 380; Shewell, 1953, Canad. Ent., 85: 465.

Lectotype ♀ (M.C.Z. Type No. 15,703).—Labelled: Pennsylvania; Loew Coll.; *virens* m. The paratype, labelled only "Loew Coll.," is in better condition. The head of the named specimen is partially crushed and it is slightly smaller than the paratype (2.75 vs. 3 mm. in wing length), but I consider both to be of the same species.

Genus **Ophiomyia** Braschnikov

Ophiomyia buscki (Frost), new combination, new emendation

Agromyza buskei Frost, 1936, Ann. Ent. Soc. Amer., 29: 315.
Melanagromyza buskei (Frost): Frick, 1952, Univ. Calif. Publ. Ent., 8: 378.

Holotype ♂ (U.S.N.M. Type No. 62,971).—Corozal, Panama Canal Zone, III–1–1911 (Aug. Busck). This species, obviously named for August Busck, was misspelled twice in the original description. This is therefore an invalid original spelling (Heming, 1953, par, 71 (1) (b) (i), pg. 44) and the name is hereby emended to *buscki*. Previously placed into *Melanagromyza* on the basis of the description, this species belongs in *Ophiomyia*.

Ophiomyia fasciculata (Malloch)

Agromyza fasciculata Malloch, 1934, Dipt. Patag. S. Chile, 6: 479.
Ophiomyia fasciculata (Malloch): Frick, 1952, Univ. Calif. Publ. Ent., 8: 382.

Malloch states in the original description that the holotype is in the U.S.N.M. This is an error, since the type is in the British Museum. It is rightfully British Museum property having been collected by Mr. and Mrs. Edwards, H. Oldroyd recently pointed out.

Ophiomyia lantanae (Froggatt)

Agromyza lantanae Froggatt, 1919, Agric. Gaz. N. S. W., 30: 665; Aldrich, 1923, Proc. Haw. Ent. Soc., 5: 262.
Ophiomyia lantanae (Froggatt): de Meijere, 1925, Tijdschr. Ent., 68: 253.
Agromyza curvipalpis (Zetterstedt), Melander, *p. p.*, 1913, Jour. N. Y. Ent. Soc., 21: 251.
Agromyza longicauda Curran, 1928, Insect. Puerto Rico and Virgin Is., 11: 65. New synonymy.

A holotype as such probably does not exist, since Froggatt's description was of a popular nature. Aldrich redescribed the species in 1923 from a "type" series of 46 Hawaiian specimens in the U.S.N.M. "Holotype" ♂ (U.S.N.M. Type No. 25,202).—Waianae, Oahu, Hawaii, XI–24–1921 (O. H. Swezey), ex *Lantana* berries. Type of synonym: holotype ♀ (published as "male") (A.M.N.H.).—St. Thomas, Virgin Islands, II–21–1925, R 5,006. Aldrich states in his paper that the name must be accredited to Froggatt, so he must not have considered the series before him as type material. However, the series is erroneously labelled as the type series, and is so recorded in the U.S.N.M. type book. The specimen of *longicauda* designated as a male by Curran is a female of this species. The female of *longicauda* belongs to *Melanagromyza minima*.

Ophiomyia proboscidea (Strobl)

Agromyza proboscidea Strobl, 1900, Wissensch. Mitt. Bosn. u. Herzegow., 7: 265.
Ophiomyia proboscidea (Strobl): Hendel, 1920, Arch. Naturgesch., Abt. A, 84: 129.
Agromyza oralis Frost, Ann. Ent. Soc. Amer., 29: 309.
Ophiomyia oralis (Frost): Frick, 1952, Univ. Calif. Publ. Ent., 8: 383. New synonymy.

Type of synonym: holotype ♂ (U.S.N.M. Type No. 62,973).—El Salto, Antigua, Guatamala (J. M. Aldrich). Compared with five European specimens of *proboscidea*, four reared from *Hieracium umbellatum* by E. M. Hering.

Ophiomyia texana (Malloch)

Agromyza texana Malloch, 1913, Ann. Ent. Soc. Amer., 6: 319; Frick, 1952, Univ. Calif. Publ. Ent., 8: 383.

Holotype ♂ (U.S.N.M. Type No. 15,582).—Brownsville, Texas, I–27–1909 (McMillan and Marsh), ex *Roripa* sp. This species is very close to, but not the same as *O. proboscidea*, as Hendel (1931) and Frick have stated.

Genus **Tylomyza** Hendel

Tylomyza nasuta (Melander)

Agromyza nasuta Melander, 1913, Jour. N. Y. Ent. Soc., 21: 260; Malloch, 1924, Canad. Ent., 56: 192.
Agromyza curvipalpis (Zetterstedt), Melander, *p. p.*, 1913, Jour. N. Y. Ent. Soc., 56: 251.
Agromyza simplex (Loew), Melander, *p. p.*, 1913, Jour. N. Y. Ent. Soc., 56: 252.
Agromyza youngi Malloch, 1914, Ent. News; 25: 312.
Ophiomyia madizina Hendel, 1920, Arch. Naturgesch., Abt. A, 84: 130; Malloch, 1924, Canad. Ent., 56: 192.
Tylomyza madizina (Hendel): Hendel, 1931, *in* Lindner, Die Flieg. Palaearkt. Reg., 59: 185; Frick, 1952, Univ. Calif. Publ. Ent., 8: 385. New synonymy.

Lectotype ♂ (A.L.M.).—Kamiac Butte, Washington, VI–1–1912; Types of synonyms: *youngi*, holotype ♂ (N.Y.S.M.).—Albany, New York, IV–28–1913 (D. B. Young), No. a2,391; *pinguis*, syntype ♂ (U.S.N.M. Cotype No. 28, 432).—no data on pin. Melander's species is not *pinguis* as Hendel (1920, 1931) and Frick (1952) thought. Melander's description does not mention the three pairs of dorsocentrals that characterize this species. Four of Strobl's specimens from Styria in Melander's collection are *pinguis* and one is *nasuta*. The name *pinguis* should be removed from the American species list.

Genus **Phytobia** Lioy

Phytobia (**Phytobia**) **picta** (Coquillett), new combination

Agromyza picta Coquillett, 1902, Jour. N. Y. Ent. Soc., 10: 188.
Agromyza dorsocentralis Frost, 1936, Ann. Ent. Soc. Amer., 29: 307.
Phytobia (*Phytobia*) *dorsocentralis* (Frost): Shewell, 1953, Canad. Ent., 85: 468. New synonymy.

Holotype ♀ (U.S.N.M. Type No. 6,655).—Frontera, Tabasco, Mexico, III–5–? (Townsend). Type of synonym: holotype ♂ (A.M.N.H.).—Patilla Point, Panama Canal Zone, I–15–1929 (C. H. Curran). Malloch (1913) synonymized *picta* with *xanthophora* Schiner, 1868. Schiner's specimens are not in the Naturhistorisches Museum at Vienna, according to Dr. Max Beier, nor could Dr. Harald Schweiger locate them in the Niederösterreichischen Landesmuseum, also at Vienna. In the absence of any information concerning *A. xanthophora*, I do not accept the synonymy proposed by Malloch and followed by Frick (1952).

Phytobia (**Phytobia**) **pruni** (Grossenbacher)

Agromyza pruni Grossenbacher, 1915, Bull. Torrey Bot. Club, 42: 235; Malloch, 1915, Ill. Nat. Hist. Survey Bull. 11: 349.
Phytobia (*Phytobia*) *pruni* (Grossenbacher): Frick, 1952, Univ. Calif. Publ. Ent., 8: 391.
(?) *Agromyza nigripes* (Meigen), Melander, 1913, Jour. N. Y. Ent. Soc., 21: 254.

There are no specimens of this species in the museums I visited nor are there any at Cornell's Ithaca campus, according to Dr. H. Dietrich. Malloch promptly redescribed the species based upon material obtained from Grossenbacher.

Melander's specimen does not completely fit Malloch's description but is probably the same species.

Phytobia (Phytobia) setosa (Loew), new combination

Agromyza setosa Loew, 1869, Berl. Ent. Zeitschr., 13: 45; Shewell, 1953, Canad. Ent., 85: 463.
Agromyza aceris Greene, 1917, Jour. Agric. Res., 10: 13; Malloch, 1918, Canad. Ent., 50: 316.
Phytobia (Phytobia) aceris (Greene): Frick, 1952, Univ. Calif. Publ. Ent., 8: 390. New synonymy.

Holotype ♂ (M.C.Z. Type No. 13,442).—Labelled: D. C.; Loew coll.; *setosa* m. Type of synonym: holotype ♀ (U.S.N.M. Type No. 21,062).—Chain Bridge, Virginia, IV–26–1916 (C. T. Greene), ex *Acer rubrum*. Malloch and others were apparently misled by Loew's failure to note the reddish color on the lower half of the frontal vitta. Malloch (1918) published keys to the species having the frons yellow or reddish and omitted *setosa*.

Phytobia (Cephalomyza) auriceps (Melander), new combination

Agromyza auriceps Melander, 1913, Jour. N. Y. Ent. Soc., 21: 262; Frick 1952, Univ. Calif. Publ. Ent., 8: 374.

Lectotype ♀ (A.L.M.).—Moscow Mtn., Idaho, VII–6–1912. A study of the types showed that this species does not belong in *Agromyza sens. str.* The best specimen of the series was chosen for the lectotype.

Phytobia (Cephalomyza) indecisa (Malloch), new combination

Agromyza indecisa Malloch, 1913, Ann. Ent. Soc. Amer., 6: 292; Frick, 1952, Univ. Calif. Publ. Ent., 8: 397; Shewell, 1953, Canad. Ent. 85: 468.

Holotype ♀ (U.S.N.M. Type No. 15,568).—Las Vegas Mtn., New Mexico, VI–1901 (T. D. A. Cockerell), 11,000 feet elevation. This species belongs to this subgenus even though the gena posteriorly is one-half the eye height. Hendel (1931) and Frick (1952) gave the genal height as two-thirds that of the eye but this figure has been reduced as the concept of *Cephalomyza* has enlarged.

Phytobia (Pöemyza) angulata (Loew)

Agromyza angulata Loew, 1869, Berl. Ent. Zeitschr., 13: 47; Melander, 1913, Jour. N. Y. Ent. Soc., 21: 254.
Phytobia (Poemyza) angulata (Loew): Shewell, 1953, Canad. Ent., 85: 466.
Agromyza neptis Loew, 1869, Berl. Ent. Zeitschr., 13: 50; Frick, 1952, Univ. Calif. Publ. Ent., 8: 373.
Phytobia (Poemyza) neptis (Loew): Shewell, 1953, Canad. Ent., 85: 468. New synonymy.
Dizygomyza semiposticata Hendel, 1920, Arch. Naturgesch., Abt. A, 84: 131. New synonymy.
Agromyza cinereifrons Frost, 1931, Canad. Ent., 63: 276. New synonymy.

Lectotype ♀ (M.C.Z. Type No. 13,434).—Labelled: Pennsylvania; Loew coll.; *angulata* m. Types of synonyms: *neptis*, lectotype ♀ (M.C.Z. Type No. 13,440).—Labelled: D. C.; No. 53; Loew coll.; *neptis* m.; *semiposticata*, syntype ♀ (U.S.N.M. Cotype No. 28,433); *cinereifrons*, holotype ♂ (U.S.N.M. Type No. 62,963).—Florida, New York, VII–7–1917 (S. W. Frost). There is some variation in the relative lengths of the two sections of vein M_3+_4. The ratio is about 1:1 in the types of *angulata* and 1.2 and 1.3:1 for *neptis*, the penultimate being the longer. In other specimens, *semiposticata* and a paratype of *cincereifrons*, this ratio is 0.8 and 0.9:1, the penultimate being the shorter. Aside from this variation, I could find no differences between the various specimens. Hendel (1931) mentions the variation in the relative lengths of M_3+_4 of *semiposticata* in Europe.

Phytobia (Pöemyza) muscina (Meigen)

Agromyza muscina Meigen, 1830, Syst. Beschr. Bekann. Eur. Zweifl. Insect., 6: 177.
Phytobia (Pöemyza) muscina (Meigen): Frick, 1952, Univ. Calif. Publ. Ent., 8: 392.
Agromyza marginata Loew, 1869, Berl. Ent. Zeitschr., 13: 49.
Phytobia (Dizygomyza) marginata (Loew): Frick, 1952, Univ. Calif. Publ. Ent., 8: 396.
Phytobia (Pöemyza) marginata (Loew): Shewell, 1953, Canad. Ent., 85: 468. New synonymy.
Agromyza superciliosa (Zetterstedt), Melander, 1913, Jour. N. Y. Ent. Soc., 21: 256.

Type of synonym: holotype ♂ (M.C.Z. Type No. 13,438).—Labelled: "Loew coll.; *marginata* m." *A. marginata* Loew and Malloch and *superciliosa* Melander unmistakably belong to this distinctive Holarctic species.

Phytobia (Icteromyza) capitata (Zetterstedt)

Agromyza capitata Zetterstedt, 1848, Dipt. Scand., 7: 2750.
Dizygomyza (Icteromyza) capitata (Zetterstedt): Hendel, 1931, *in* Lindner: Die Flieg. Palaearkt. Reg., 59: 52.
Phytobia (Icteromyza) capitata (Zetterstedt): de Meijere, 1955, Tijdschr. Ent., 98: 6.
Agromyza genualis Melander, 1913, Jour. N. Y. Ent. Soc., 21: 261.
Phytobia (Icteromyza) genualis (Melander): Frick, 1952, Univ. Calif. Publ. Ent., 8: 393. New synonymy.
Agromyza coloradensis Malloch, 1913, Ann. Ent. Soc. Amer., 6: 297. New synonymy.

Types of synonyms: *genualis*, lectotype ♀ (A.L.M.).—Mt. Constitution, Orcas Island, Washington, VIII–7–1905; *coloradensis*, holotype ♂ (U.S.N.M. Type No. 15,570).—Florissant, Colorado, VI–27–1907 (S. A. Rohwer). One of the two females from Mt. Constitution was chosen for the lectotype. Compared with four specimens of *capitata* received from Prof. E. M. Hering.

Phytobia (Icteromyza) pollinosa (Melander), new combination

Agromyza pollinosa Melander, 1913, Jour. N. Y. Ent. Soc., 21: 263; Frick, 1952, Univ. Calif. Publ. Ent., 8: 375.

Lectotype ♂ (A.L.M.).—Sitka, Alaska, VII–16–1907 (W. T. Shaw). I chose for the type the specimen that has its antennae, although neither specimen is in really good condition.

Phytobia (Calycomyza) ipomoeae (Frost), new emendation

Agromyza ipomaeae Frost, 1931, Ent. News, 42: 74.
Phytobia (Calycomyza) ipomaeae (Frost): Frick, 1952, Univ. Calif. Publ. Ent., 8: 395.

Holotype ♂ (U.S.N.M. Type No. 62,961).—Rio Piedras, Puerto Rico, VI–20–1930 (M. D. L.), ex sweet potato leaves. This species, obviously named for the genus *Ipomoea*, was misspelled in the original description. This is therefore an invalid original spelling (Heming, 1953, par. 71 (1) (b) (i), pg. 44) and the name is hereby emended to *ipomoeae*.

Phytobia (Trilobomyza) varia (Melander), new combination

Agromyza varia Melander, 1913, Jour. N. Y. Ent. Soc., 21: 264.
Phytobia (? Subgenus) *varia* (Melander): Frick, 1952, Univ. Calif. Publ. Ent., 8: 397.

Holotype ♀ (A.L.M.).—Moscow Mtn., Idaho, VI–12–1910. A study of the type showed that the species is a typical *Trilobomyza*.

Phytobia (Praspedomyza) correntosana (Malloch), new combination

Agromyza correntosana Malloch, 1934, Dipt. Patag. S. Chile, 6: 478.
Phytobia (? Subgenus) *correntosana* (Malloch): Frick, 1952, Univ. Calif. Publ. Ent., 8: 397.

Holotype ♀ (U.S.N.M. Type No. 50,299).—Correntoso, Rio Negro, Argentina, XI–1926 (R. and E. Shannon). The holotype is not a male, as stated by Malloch, and is a typical *Praspedomyza*.

Phytobia (Dizygomyza) thompsoni Frick

Agromyza magnicornis (Loew), Coquillett, 1907, *in* Thompson, 1907, Psyche, 14: 74.
Agromyza laterella (Zetterstedt), Malloch, *p. p.*, 1913, Ann. Ent. Soc. Amer., 6: 300; Claassen, 1918, Ann. Ent. Soc. Amer., 11: 9.
Phytobia (Dizygomyza) thompsoni Frick, 1952, Univ. Calif. Publ. Ent., 8: 396.

Lectotype ♂ (U.S.N.M.).—Worcester, Massachusetts, ex gall on Iris. Frick (1952) gave the species a new name but lacked specimens for a type designation. This specimen is apparently from Thompson's original material (Malloch, 1913) as it was identified by Coquillett.

Genus **Liriomyza** Mik

Liriomyza chlamydata (Melander), new combination

Antineura chlamydata Melander, 1913, Jour. N. Y. Ent. Soc., 21: 250.
Haplomyza chlamydata (Melander): Hendel, 1914, Ent. Mitt., 3: 73; Frick, 1952, Univ. Calif. Publ. Ent., 8: 410.

Holotype ♂ (A.L.M.).—Oroville, Washington, V–1–1912. Although crossvein m-m is lacking in the wing, *chlamydata* belongs under *Liriomyza*. The differences between the genera will be clarified in the forthcoming synopsis.

Liriomyza gayi (Porter)

Agromyza gayi Porter, 1915, Bol. Mus. Nac. Chile, 8: 56; Malloch, 1934, Dipt. Patag. S. Chile, 6: 472.
Liriomyza gayi (Porter): Frick, 1952, Univ. Calif. Publ. Ent., 8: 403.

There is a specimen in the U.S.N.M.: Paratype ♂ (U.S.N.M. Paratype No. 49,380).—Ancud. Isla Chiloe, Chile, XII–1926 (R. and E. Shannon); labelled: "*Agromyza gayi* paratype, det. J. R. Malloch." These data were entered in the U.S.N.M. type book in 1933 for a paratype of *A. gayi*. This specimen cannot be an authentic paratype, since the species was described in 1915 and this specimen was not collected until 1926. Malloch does not mention type material of this species in his 1934 paper.

Liriomyza lima (Melander)

Agromyza lima Melander, 1913, Jour. N. Y. Ent. Soc., 21: 265.
Liriomyza lima (Melander): Frick, 1952, Univ. Calif. Publ. Ent., 8: 404.
Agromyza holti Malloch, 1924, Canad. Ent., 56: 191.
Liriomyza holti (Malloch): Frick, 1952, Univ. Calif. Publ. Ent., 8: 403. New synonymy.

Lectotype ♂ (A.L.M.).—Moscow Mtn., Idaho, VII–6–1912 (A. L. Melander). Type of synonym: holotype ♀ (U.S.N.M., without type No.).—Ardmore, South Dakota, VIII–13–1915 (E. G. Holt). I chose the best male for the lectotype, because that is the sex described by Melander. This is a distinctive species and there is no doubt that Malloch's type, although in very poor condition, is the same as *lima*.

Liriomyza melampyga (Loew)

Agromyza melampyga Loew, 1869, Berl. Ent. Zietschr., 13: 48.
Liriomyza melampyga (Loew): Frick, *p. p.*, 1952, Univ. Calif. Publ. Ent., 8: 404; Shewell, 1953, Canad. Ent., 85: 467.
Agromyza flaviventris Johnson, 1902 (*nec* Strobl, 1898), Canad. Ent., 34: 242.

Lectotype ♀ (M.C.Z. Type No. 13,439).—Labelled: "Loew coll.; *melampyga* m." Type of synonym: 2 ♀♀ syntypes (M.C.Z. Type No. 7,895).—Niagara Falls, New York, VI–1923 (C. W. Johnson). Loew's collection contains two specimens. The male is headless while the female is in good condition. This species does not include specimens reared from *Plantago* or *Philadelphus* as Malloch (1913) and others have believed.

Liriomyza pacifica (Melander)

Agromyza pacifica Melander, 1913, Jour. N. Y. Ent. Soc., 21: 264.
Liriomyza pacifica (Melander): Frick, 1952, Univ. Calif. Publ. Ent., 8: 404.
Agromyza longispinosa Malloch, 1913, Ann. Ent. Soc. Amer., 6: 276.
Liriomyza longispinosa (Malloch): Shewell, 1953, Canad. Ent., 85: 467.

Lectotype ♀ (A.L.M.).—Bellingham, Washington, VII–30–?. Type of synonym: holotype

♂ (U.S.N.M. Type No. 15,558).—Bear Lake, British Columbia, VII–20–1903 (R. P. Currie). The specimen of *pacifica* in the best condition was chosen for the type.

Liriomyza phaseolunata (Frost)

Agromyza phaseolunata Frost, 1943, Jour. N. Y. Ent. Soc., 51: 256.
Liriomyza phaseolunata (Frost): Frick, 1952, Univ. Calif. Publ. Ent. 8: 404.

The holotype is apparently lost but there are three paratypes in the S. W. Frost collection, as follows: 2 ♂♂, 1 ♀ (S.W.F.).—Bridgetown, New Jersey, VIII–24–1942, ex lima bean. The type was probably sent to the U.S.N.M., because the species was described from four specimens and Drs. B. B. Pepper and J. B. Schmitt have written that specimens do not exist at Rutgers. The holotypes of two species described by Frost at the same time, *L. allia* and *subpusilla*, are in U.S.N.M. A neotype designation is not in order as the holotype may be found and *phaseolunata* is easily distinguished from *trifolii*, the most similar species.

Liriomyza trifolii (Burgess)

Oscinis trifolii Burgess, 1880, U. S. Dept. Agric. Ann. Rpt., 1879: 201.
Agromyza trifolii (Burgess): Coquillett, 1898, U. S. Dept. Agric., Div. Ent. Bull. 10: 78.
Liriomyza trifolii (Burgess): Frick, 1952, Univ. Calif. Publ. Ent., 8: 405; 1953, Canad. Ent., 85: 72.
Liriomyza congesta (Becker), 1903, Mitt. Zool. Mus., 2: 190; Frick, 1953, Canad. Ent., 85: 72. New synonymy.

The type of *trifolii* could not be found in the U.S.N.M. although Coquillett mentioned the types of Burgess' species when he placed the species into *Agromyza*. The descriptions of adult and mine are brief but are adequate to characterize the species. A neotype is not needed because *trifolii* is a rather distinctive species and the leaf mines made by the larvae are particularly diagnostic. Frick (1953) considered *trifolii* a secondary homonym. This is in error as Frick did not consider *trifolii* (Burgess) and *Agromyza trifolii* Kaltenbach, 1874, as congeneric at the time that the homonymy was discovered.

Genus **Metopomyza** Enderlein

Metopomyza interfrontalis (Melander), new combination

Agromyza interfrontalis Melander, 1913, Jour. N. Y. Ent. Soc., 21: 263.
Liriomyza interfrontalis (Melander): Frick, 1952, Univ. Calif. Publ. Ent. 8: 403.

Holotype ♀ (A.L.M.).—Tacoma, Washington, VIII–27–1911. This is the first record of a species of *Metopomyza* occurring in North America.

Genus **Haplomyza** Hendel

Haplomyza togata (Melander)

Antineura togata Melander, 1913, Jour. N. Y. Ent. Soc., 21: 250.
Haplomyza togata (Melander): Hendel, 1914, Ent. Mitt., 3: 73.
Phytomyza minuta Frost, *p. p.*, 1924, Cornell Univ. Agric. Expt. Sta. Mem., 78: 86; Frick, 1953, Canad. Ent., 85: 73.

Lectotype ♂ (A.L.M.).—Pullman, Washington, V–12–1912. I felt that the male was the better of the two syntypes and herein designate it the type.

Genus **Phytoliromyza** Hendel

Phytoliriomyza arctica (Lundbeck)

Agromyza arctica Lundbeck, 1900, Vid. Medd., 5: 304.
Dizygomyza (Icteromyza) arctica (Lundbeck): Hendel, 1931, *in* Lindner; Die Flieg. Palaearkt. Reg., 59: 57.
Phytobia (Icteromyza) arctica (Lundbeck): Frick, 1952, Univ. Calif. Publ. Ent., 8: 393.
Phytoliriomyza arctica (Lundbeck): Shewell, 1953, Canad. Ent., 85: 469.

Lectotype ♂ (Universitetets Zoologiske Museum, Copenhagen).—Ipiutat, Greenland, VI–9–1898. Dr. Tuxen kindly lent me two syntypes for a lectotype designation. Both the male and female were in excellent condition. A male and a female syntype are in the U.S.N.M., received from the Zoologiske Museum. Each has Greenland Acc. No. 71,429 (Lundbeck) on the label.

Phytoliriomyza immaculata (Coquillett), new combination

Odinia immaculata Coquillett, 1902, Jour. N. Y. Ent. Soc., 10: 185.
Agromyza immaculata (Coquillett): Malloch, 1913, Ann. Ent. Soc. Amer., 6: 289.
Agromyza perpusilla Meigen, Melander, *p. p.*, 1913, Jour. N. Y. Ent. Soc., 21: 257.
Phytoliriomyza perpusilla (Meigen): Frick, *p. p.*, 1952, Univ. Calif. Publ. Ent., 8: 411.

Holotype ♀ (U.S.N.M. Type No. 6,649).—Mt. Washington, New Hampshire (Mrs. A. T. Slosson). This specimen is teneral and so was easily confused with the more yellowish *perpusilla*. *P. immaculata* is the more common species in the United States. All of Melander's U. S. specimens belong to *immaculata*.

Genus **Phytagromyza** Hendel

Phytagromyza orbitalis (Melander)

Phytomyza orbitalis Melander, 1913, Jour. N. Y. Ent. Soc., 21: 271.
Phytagromyza orbitalis (Melander): Frick, 1952, Univ. Calif. Publ. Ent. 8: 416.

Lectotype ♂ (A.L.M.).—Kamiac Butte, Washington, VI–1–1912. I designated the best specimen in the series to be the type.

Genus **Phytomyza** Fallén

Phytomyza atricornis Meigen

Phytomyza atricornis Meigen, 1838, Syst. Beschr. Bekann. Eur. Zweifl. Insekt., 7: 404.
Phytomyza chrysanthemi Kowarz, 1891, Rept. N. Y. Mus., 44: 243; Smulyan, 1914, Mass. Agric. Exp. Sta. Bull. 157: 21; Frost, 1924, Cornell Univ. Agric. Exp. Sta. Mem., 78: 70.

The types of both species were studied by Hendel (1920) who made the synonymy. I compared Smulyan's specimens in Frost's personal

collection with specimens of *atricornis* from E. M. Hering. They proved to be identical. The *atricornis* of Aldrich and Frost (1924) is not *atricornis sens. str.* but a closely related species.

Phytomyza diminuta Walker, *nomen dubium*

Phytomyza diminuta Walker, 1857, Trans. Ent. Soc. London, n. ser., 4: 233.

H. Oldroyd has written (I–18–1956) that specimens of this species are not in the British Museum. Walker's brief description is inadequate to diagnose the species and since the type has apparently been lost, *diminuta* is considered a *nomen dubium*.

Phytomyza genalis Melander

Phytomyza genalis Melander, 1913, Jour. N. Y. Ent. Soc., 21: 272.

Lectotype ♂ (A.L.M.).—Chicago, Illinois. Of the two syntypes, the male is in the better condition of the two specimens.

Phytomyza ilicicola Loew

Phytomyza ilicis Loew, 1863 (*nec* Curtis, 1846), Berl. Ent. Zeitschr., 7: 54. Primary homonym.
Phytomyza ilicicola Loew, 1872 (n. n. for *P. ilicis* Loew, 1863, *nec* Curtis, 1946), Berl. Ent. Zeitschr., 16: 114.

Lectotype ♀ (M.C.Z Type No. 13,431).—Labelled only: "Loew coll." Another female bearing labels: "D. C., No. 86; Loew coll., *ilicis* m."; also an M.C.Z. type label, is headless. Therefore I chose the female possessing a head for the type. There are five more specimens in the M.C.Z. collection from the collection of Osten Sacken. Four have the label: "The larva mines the leaves of *Ilex opaca*."

Phytomyza lactuca Frost

Phytomyza lactuca Frost, 1924, Cornell Univ. Agric. Expt. Sta. Mem., 78: 85.

Although originally described from four specimens, only three can now be found in Frost's personal collection. The fourth must have gone to the U.S.N.M. although it is not recorded in the type book as are the remaining holotypes of Frost's 1924 species. No neotype designation is contemplated, since this species is rather distinctive.

Phytomyza loewii Hendel

Phytomyza clematidis Loew, 1863 (*nec* Kaltenbach, 1859), Berl. Ent. Zeitschr., 7: 55. Primary homonym.
Phytomyza loewii Hendel, 1923 (n. n. for *P. clematidis* Loew, 1863, *nec* Kaltenbach, 1859), Konowia, 2: 145.
Phytomyza nitida Melander, *p. p.*, 1913, Jour. N. Y. Ent. Soc., 21: 271.

Lectotype ♀ (M.C.Z. Type No. 13,429).—Labelled: "D. C.; No. 85; Loew coll.; *clematidis* m." Loew states that male and female were before him, but there are only two females. There are also two, male and female, collection of Osten Sacken, under this name with the label: "The larva mines the leaves of *Clematis*."

Phytomyza minuscula Goureau

Phytomyza minuscula Goureau, 1851, Ann. Soc. Ent. France, (2) 9: 153; Frick, 1952, Univ. Calif. Publ. Ent., 8: 427.
Phytomyza nitida Melander, 1913, Jour. N. Y. Ent. Soc., 21: 271. New synonymy.

Type of synonym: lectotype ♂ (A.L.M.)—Moscow Mtn., Idaho, VII–5–1912. The specimens from White Plains, New York, Troy and Bovill, Idaho, are *P. loewii*. Therefore, the type was chosen from a male and a female from Moscow Mtn. No differences could be found between the type specimens of *nitida* and specimens of *minuscula* from Europe and from North America reared from *Aquilegia* spp. and *Thalictrum* spp. (Frick, 1952).

Phytomyza plantaginis R.-D.

Phytomyza plantaginis R.-D., 1851, Rev. Mag. Zool., (2) 3: 404.
Phytomyza genualis Loew, 1869, Berl. Ent. Zeitschr., 13: 52. New synonymy.

Type of synonym: holotype ♀ (M.C.Z. Type No. 13,430).—Labelled: "Loew coll.; *genualis* m." Compared with specimens reared from *Plantago* in Europe by Hering.

Phytomyza solita Walker, *nomen dubium*

Phytomyza solita Walker, 1857, Trans. Ent. Soc. London, n. ser. 4: 232.

H. Oldroyd has written (I–18–1956) that specimens of this species are not in the British Museum. Walker's brief description is inadequate to diagnose the species and since the type has apparently been lost, *solita* is considered a *nomen dubium*.

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THE MALE, DEUTONYMPH AND PROTONYMPH OF THE MITE EUBRACHYLAELAPS CIRCULARIS (EWING) (ACARINA: LAELAPTIDAE) WITH NOTES ON MORPHOLOGICAL VARIATIONS[1]

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In his generic revision of *Eubrachylaelaps*, Furman (1955) recognized eight species. All of these were described from female specimens only. Apparently there have been few workers who have seen or recognized the males. Jameson (1950) states that he has seen the males of only two species. Strandtmann (1949) suggested that males of the genus *Haemolaelaps* seldom are found on the host, and probably are nest dwellers. It is apparent from collections made by the author and other workers that this applies to males of the genus *Eubrachylaelaps*. During a seasonal study of the ectoparasites found in the nests of the desert wood rat (Allred and Roscoe, unpublished manuscript), the author and his associates collected one nest infested with females, males, deutonymphs and protonymphs of *E. circularis*. The collection of all of these stages from a rodent nest lends support to the theory of the nest dwelling habit of this species.

The descriptions of the androtype male, plesiotype deutonymph and plesiotype protonymph follow. All measurements given are in microns. Length and width figures for the plates refer to the greatest distance exclusive of the very narrow projections such as the intercoxal projections on the holoventral plate of the male.

MALE

(Figs. 1, 2, 7, 10)

Idiosoma. 591 long, 448 wide.

Gnathosoma. Greatest width near base, 110; length from base to distal deutosternal teeth, 69. Internal middle pair hypostomal setae 26 long; external middle setae 12 long; posterior and anterior setae 24 long. Length of palps from base of gnathosoma, 194; width of first separate joint (trochanter), 31. Chelicerae without teeth; digitus fixus slender, without pilus dentilis; digitus mobilis extended into long, recurved finger, with a brush of microchaetae at its base.

Venter. Base of tritosternum 15 long; length of distal segment from base to fork, 24; length of lacinae from fork, 81; serration of lacinae continuing from tip past the fork almost to base of distal segment. Holoventral plate conspicuously reticulate, 454 long; narrowest width at level of coxae IV, 91; greatest width immediately behind coxae IV, 257; tapering abruptly posteriorly to a blunt point and enclosing the anal opening. The usual five pairs of nude setae on plate thicker than anal or accessory setae; first pair 48 long, situated on anterior edge of plate; second, fourth and fifth pairs 62 to 67 long; third pair 72 long; fifth pair situated slightly posterior to middle of coxae IV; plate with 10 accessory setae, 40 to 55 long; longest setae in center of plate. Paired anal setae 36 long, situated opposite posterior half of anal opening. Genital aperture on anterior edge of plate, projecting anteriorly into reticulate area behind tritosternum. Sternal pores slit-like; first and third pairs approximately parallel with anterior edge of plate; second pair at approximately 45° angle. Peritreme slightly sinuous, extending to anterior half of coxa II. Stigma situated between coxae III and IV. Peritremalia extends posteriorly from stigma with pore at apex of cleft; two pores in figure-eight-shaped plate near opening of cleft. Unsclerotized portion of venter with eight pairs of barbed setae, 31 to 50 long; posterior pair longest. Legs typical of the genus, with posteroventral setae of coxa III thickened into thorn-like spine; other setae of normal shape and size. Legs I and II thicker than III and IV; II and III, 346 long; I, 397 long; IV, 479 long.

Dorsum. Oval dorsal plate reticulate, conspicuously so laterally and posteriorly; covers most of dorsal surface; 555 long and 376 wide. Plate with 39 pairs of setae; anterior and marginal setae largest, 36 to 48 long; internal setae 24 to 33 long; posterior pair 81 long; marginal setae barbed. Two pairs of sensory spots on posterior third of plate; 1 pair of slit-like pores at anterior end, and 13 pairs of circular pores on plate. Unsclerotized portion with 11 pairs of barbed setae, 36 long.

DEUTONYMPH

(Figs. 4, 5, 9, 12)

Idiosoma. 530 to 622 long, 397 to 561 wide.

Gnathosoma. Greatest width midway, 88 to 120. Internal middle pair of hypostomal setae 36 to 40 long; external middle setae 12 to 14 long; posterior setae 24 to 31 long; anterior setae 16

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to 24 long. Palps, from base of trochanter, 96 to 100 long. Chelicerae chelate. Digitus fixus two-thirds as long as digitus mobilis; a single microseta at its base; with one terminal and one subterminal tooth; pilus dentilis 10 long and slightly enlarged near distal end. Digitus mobilis with one terminal and two subterminal teeth; with brush of basal microchaetae.

Venter. Base of tritosternum 18 to 21 long; length of distal segment from base to fork of lacinae, 16 to 21; length of lacinae from fork, 89; serration of lacinae extending from tip almost to base of distal segment. Sternal plate reticulate, most conspicuously at anterior edge; 235 to 295 long, ending at posterior edge coxae IV; 98 to 127 wide at level of coxae III; narrowed posteriorly to rounded tip 40 wide; with four pairs of setae of about equal thickness but variable in length, first pair 36 to 45 long; second pair 40 to 50 long; third pair 43 to 52 long; fourth pair 31 to 40 long. Three pairs of sternal pores; first pair directly posterior to first sternal setae and parallel to anterior edge plate; second pair at 45° angle on edge of plate directly posterior to second sternal setae; third pair at right angles to first pair, situated at edge of plate and directly anterior to fourth sternal setae. Anal plate broadly pyriform, rounded anteriorly, slightly longer than broad; 96 to 108 long, 86 to 98 wide; anal opening situated mostly in anterior half of plate; paired anal setae 26 to 33 long, situated opposite posterior half of anus; postanal seta 33 to 43 long, about equal to thickness of paired setae. Peritreme sinuous, extending from anterior edge of coxa IV to posterior fourth of coxa I. Unsclerotized portion of venter with

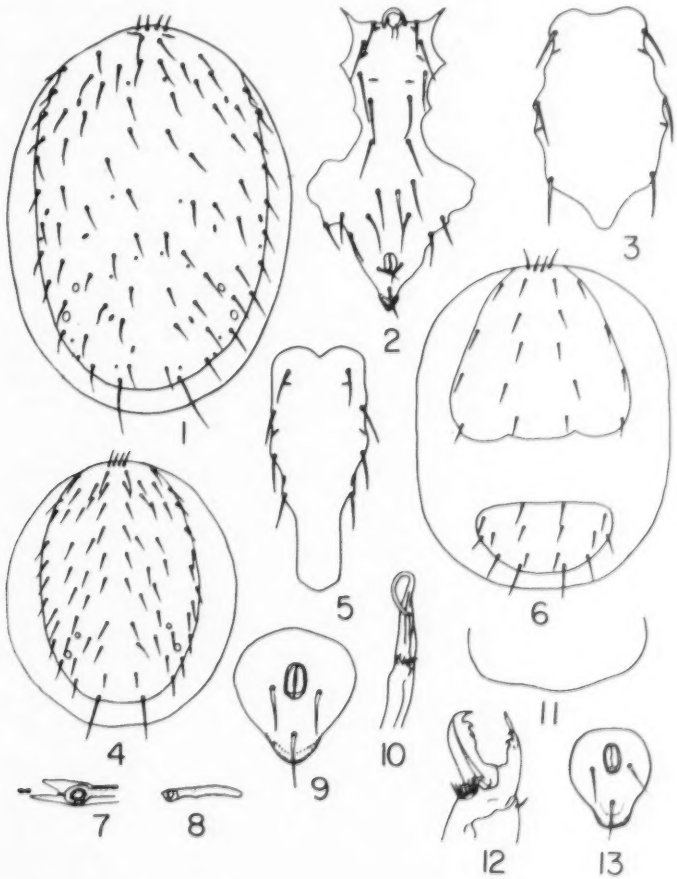

FIG. 1. Dorsal plate of male. FIG. 2. Holoventral plate of male. FIG. 3. Holoventral plate of protonymph. FIG. 4. Dorsal plate of deutonymph. FIG. 5. Holoventral plate of deutonymph. FIG. 6. Dorsal plates of protonymph. FIG. 7. Peritreme of male. FIG. 8. Peritreme of protonymph. FIG. 9. Anal plate of deutonymph. FIG. 10. Left chela of male, dorsal view. FIG. 11. Posterior edge of anterior dorsal plate of protonymph. FIG. 12. Right chela of deutonymph. FIG. 13. Anal plate of protonymph.

13 to 15 pairs of setae, 16 to 48 long, the posterior pair longest; marginal setae thicker and barbed. Legs I and II thicker than III and IV; legs I and III, 306 long; leg II, 255 long; leg IV, 428 long. Coxal setae normal, about equal in size except for the slightly smaller seta on coxa IV.

Dorsum. Oval dorsal plate reticulate; covering most of dorsal surface; 499 long, 321 wide; 39 pairs setae, 21 to 40 long; anterior and posterior internal setae about equal in size; marginal setae larger and barbed; most posterior pair setae 57 long; posterior marginal setae barbed. Two pairs sensory spots on posterior third of plate not as distinct as in male or female. Unsclerotized portion of dorsum with 13 pairs of nude and barbed setae 36 to 45 long.

PROTONYMPH

(Figs. 3, 6, 8, 11, 13)

Idiosoma. 387 to 530 long, 285 to 408 wide.

Gnathosoma. Greatest width near base, 64 to 105. Internal middle pair hypostomal setae 31 to 33 long; anterior setae 14 to 21 long. Length of palps from base to trochanter, 79 to 84. Chelicerae chelate, similar to deutonymph. Digitus fixus two-thirds as long as digitus mobilis; a single microseta at its base; with one terminal and one subterminal tooth. Digitus mobilis with one terminal and two subterminal teeth; with straight, slender pilus dentilis, 15 long, not enlarged near tip; with brush of basal microchaetae.

Venter. Base of tritosternum 16 to 19 long; length of distal segment from base to fork of lacinae, 16 to 19; length of lacinae from fork, 76; serration of lacinae extending from near tip almost to base of distal segment. Sternal plate reticulate, 165 to 177 long, ending at about middle of coxae IV; 108 to 110 wide at level of anterior edge coxae III; narrowed abruptly posteriorly to a rounded point; with three pairs of subequal setae, 31 long. Two pairs of sternal pores; first pair slightly less than 45° angle with anterior edge of plate, situated at edge of plate directly posterior to first sternal setae; second pair at about the same angle as first pair, situated on edge of plate directly posterior to second sternal setae. Anal plate pyriform, rounded anteriorly, slightly longer than broad; 74 to 88 long, 67 to 74 wide; anal opening situated mostly in anterior half of plate; paired anal setae 24 to 31 long, situated opposite posterior edge of anus; postanal seta 28 to 33 long, about equal in thickness to paired setae. Peritreme short, almost straight, barely extending past posterior edge coxa III. Unsclerotized portion of venter with three pairs of nude setae, 24 to 31 long, situated anterior to anal plate, and one barbed pair 36 to 43 long, situated opposite posterior edge anal plate. Legs I and II slightly thicker than III and IV; leg I, 271 long; legs II and III, 247 long; leg IV, 323 long. Coxal setae normal, about equal in size except for the slightly smaller seta of coxa IV.

Dorsum. Two dorsal plates. Anterior plate reticulate, extending from anterior edge to about middle of dorsum; posterior edge almost truncate, sometimes projected medially into a slightly rounded point, or with two slight concavities; widest portion at level of coxae III and IV; 256 to 276 long, 247 to 268 wide; with 11 pairs of nude setae 16 to 28 long. Posterior plate reticulate, occupying posterior fourth of dorsum; modified oval in shape, with broadly rounded posterior edge and almost truncate anterior edge; 124 long and 206 wide; with 8 pairs of setae 16 to 43 long; lateral and posterior marginal setae fimbriate, larger than internal nude setae; posterior pair of setae largest on plate. Unsclerotized portion of dorsum with 10 pairs of nude setae, 19 to 28 long.

HABITAT AND LOCALITY

Androtype. Collected October 6, 1954 by D. M. Allred and E. J. Roscoe from a nest of a desert wood rat, *Neotoma lepida lepida* Thomas,

TABLE 1

SOME DIFFERENCES BETWEEN *Eubrachylaelaps circularis* FOUND IN THREE GEOGRAPHIC AREAS IN UTAH

	AREAS 1 AND 2	AREA 3
Amount of dorsal surface which dorsal plate covers	More than on mites from Area 3	Less than on mites from Areas 1 and 2
Number setae on dorsal plate	Usually 36	Usually 39
Fourth seta on inner row of ventral setae	In line	Offset
Outer pair of accessory metapodal plates	Moderately sclerotized and distinct	Lightly sclerotized and indistinct
General body setae	More slender than on mites from Area 3	Thicker than on mites from Areas 1 and 2
Seasonal occurrence and reproductive period	Every month	April to September

located in a juniper tree in the south end of the Cedar Mountains, approximately 35 miles south of U. S. Highway 40–50, and 30 miles west of Clover, Tooele County, Utah. Elevation 4700 feet. Deposited in the collection of the U. S. National Museum.

Paratypes and *Plesiotypes*. Nine males, 30 deutonymphs and 7 protonymphs with same data as the androtype. In the collections of the author, R. W. Strandtmann, F. da Fonseca, D. P. Furman, British Museum of Natural History, Muséum National d'Histoire Naturelle, University of Utah Entomology Museum, and U. S. National Museum.

DISCUSSION

Furman (1955: 57) states that in *E. circularis*, "several characters previously believed constant and reliable at the species level have been observed to exhibit considerable intraspecific variation, in part associated with geographical distribution." He mentioned specifically the length and width ratios of the sternal plate, anal plate, and anal setae.

During a study of the mites found on mice of the genus *Peromyscus* in Utah (Allred, unpublished manuscript), the author found the geographic distribution of *E. circularis* to be rather irregular. Although it is believed that this species is statewide in distribution, it was not found on white-footed mice in the area between north latitudes 38° 30′ and 39° 30′ across the entire state, even though systematic collections were made in this area. This species of mite was found in three distinct geographic areas: (1) a northern area which consists of the Wasatch and Uinta Mountain provinces and adjacent regions; (2) a central area which consists of the Southern High Plateau regions; and (3) a southern area comprising the regions of the Virgin, San Juan and Colorado rivers (See Durrant, 1952: 480). Mites that were collected in these different geographic localities differed slightly in certain structures, seasonal occurrence, and reproductive habits. These differences are shown in Table I. Although mites from areas 1 and 2 are similar morphologically, each group was geographically isolated from the other. Because of the morphological variation that exists within this species, mites of these three separate groups can not be separated morphologically as distinct species or subspecies. It appears that because of their similarity, the apparent break in their distribution has not been of sufficient time to allow major differences which permit mites from these areas to be separated on a morphological basis. However, the variations in structure and the differences in the geographic distribution, seasonal occurrence, and reproductive periods of these mites probably indicate incipient subspecies.

Furman (1955) suggests that the adults of *Eubrachylaelaps* are not normally blood feeders. An examination of 633 mites of the species *E. debilis* collected from white-footed mice by the author showed many of them to contain blood (Allred, unpublished manuscript). Using the Allmar feeding technique (Allred and Marchette, unpublished manuscript), mites of the species *E. circularis* fed readily in the laboratory when blood was offered to them. The mites fed frequently, but took very little blood. Those that did not feed died within a day or two after they were collected. Others that fed daily or every second day remained alive for as long as two weeks. Although these observations are not conclusive concerning the feeding habits of *Eubrachylaelaps*, they suggest that these mites are blood feeders.

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NOTICE TO AUTHORS

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BOOK NOTICES

AQUATIC INSECTS OF CALIFORNIA. Edited by Robert L. Usinger. University of California Press, Berkeley 4, California, 1956. 508 pp. $10.00.

For the last two decades students of aquatic insects have collected West Coast material of great interest, much of it new to science. This knowledge has in the main been unincorporated into comprehensive studies and at the same time has made many of the older comprehensive books out-of-date for the western area. In one bold stroke Dr. Usinger and his collaborators have brought this new material together, integrated it with the old, and produced a book which is not only indispensable in studying aquatic insects in the West but is one of the most important and helpful publications of the decade on North America entomology.

This book contains an amazing amount of information. Its 508 pp. are 8½ x 11", double column, equalling from 1200–1500 pages of a conventional single column book. Sixteen authors have contributed chapters. In this review authors of specific sections are indicated in parentheses.

Introductory chapters contain (1) an outline of much useful general information including ecological aquatic communities in California, an outline of many of the ecological principles governing aquatic insects, outlines of mosquito and gnat control, and important aspects of stream and lake management (R. L. Usinger); (2) a description of collecting and study techniques with special reference to aquatic insects (J. D. Lattin); and (3) a brief morphological outline and key to the orders of aquatic insects (W. C. Bentinck).

The principal portion of the book consists of taxonomic treatments of the aquatic members of 13 insect orders. For each there are excellent accounts of the biology of the group and wherever possible keys have been provided to the nearctic genera of adults, nymphs, larvae, and pupae, and to the species occurring in California.

The chapter on aquatic Collembola (D. B. Scott), Orthoptera (I. LaRivers), Neuroptera (H. P. Chandler), and Hymenoptera (K. S. Hagen) embrace only small scattered groups of species, and these chapters will be of interest chiefly to the limnologist interested in identifying all members of aquatic communities. The small chapter on aquatic Hymenoptera contains no taxonomic material but brings together accounts of the peculiar habits of these intriguing forms.

The chapters on Megaloptera (H. P. Chandler) and aquatic Lepidoptera (W. H. Lange, Jr.) are also small but contain much original and welcome information on the taxonomy and biology of the two groups. The aquatic Lepidoptera in this area comprise only the pyralid subfamily Nymphulinae plus one other pyralid genus *Acentropus*.

The large bulk of the book treats the entirely aquatic orders Ephemeroptera (W. C. Day), Odonata (A. E. Pritchard and R. F. Smith), Plecoptera (S. G. Jewett, Jr.), and Trichoptera (D. G. Denning), plus the aquatic families or genera of the Hemiptera (R. L. Usinger), Coleoptera (H. B. Leech and H. P. Chandler), and Diptera (W. W. Wirth and Alan Stone). For the Tipulidae and a few small families in this last chapter the California species are listed but not keyed. The chapter is nevertheless the largest in the book. In each group California has a large and varied fauna, hence these accounts are of the greatest value both to specialists in all parts of the country and to West Coast students wishing to begin serious study on the western fauna. Many of the chapters come close to completeness also for the species of several other western states.

The chapters on the aquatic orders (Ephemeroptera, Odonata, Plecoptera, and Trichoptera) are especially well illustrated as regards characters diagnosing both genera and species. Illustrations have been assembled from many sources and new drawings added (especially a fine set in the Odonata by Celeste Green). The other large chapters are also well illustrated but to a more modest degree. Welcome additions to the literature are the many new toto views of aquatic Hemiptera from the pen of Arthur Smith.

The book ends with a helpful glossary (W. C. Bentinck) and index. The styling and planning of the entire book are excellent, the only disappointment being the poor reproduction obtained on some of the borrowed illustrations. It should be noted, however, that only poor copy was available for many originally made on stipple board for the Needham and Classen stonefly monograph.

One of the most interesting general features of the book is the fine accumulation of adult and larval pictures of many odd families such as the Hydroscaphidae and Eurystethidae in the beetles and the Deuterophlebiidae, Empididae, and Canaceidae in the flies.

The authors and the University of California Press deserve congratulations for producing this fine book indispensable to all interested in aquatics and of great interest also to entomologists with a proclivity for the natural history of insects.

H. H. Ross.

A CLASSIFICATION OF THE FIRST INSTAR LARVAE OF THE MELOIDAE (COLEOPTERA), by J. W. MacSwain. University of California Publications in Entomology, University of California Press, vol. 12, iv plus 181 pp., 29 pls. 1956. $3.00.

The title is perhaps an understatement of the scope of the paper, for in actuality this work represents a carefully analyzed account of both the phylogeny and the classification of the Meloidae of the world. Furthermore, while the author's primary source of data was a comparative study of the morphology of the first stage larvae, information pertaining to the morphology of the adults and especially biology was integrated and temperately synthesized wherever possible. Since the author's ideas concerning the systematics of the family were based on all these lines of evidence, there is little doubt but that this definitive paper will represent the basic framework of the classification of the Meloidae for years to come, in spite of the fact that small changes will become advisable when more biological data are uncovered, when larvae of other species are collected, and when the adults are more completely studied.

The general outine of the paper is as follows: After the introduction and acknowledgements, the author briefly but concisely presents the history of the biological and systematic work pertaining to the larvae. He next treats the known biology of the members of the family, summarizing the data at the end in the form of comparative biological diagnoses of three of the five subfamilies. Little is known of the other two subfamilies, which, however, are small. This is followed by a discussion of the morphology of the first instars, with particular reference to an evaluation of the characters of systematic use. The last part of the paper, dealing with systematics of the family, is by far the largest, occupying 127 pages. It is introduced by a treatment of the phylogeny, in which the author's reasons, both biological and morphological, for dividing the family into five subfamilies are presented and discussed. The paper then provides, in a strictly taxonomic arrangement, an account of the subfamilies, tribes, genera, and species based upon the known first stage larvae. This section includes both keys and comparative descriptions, and lists as well the geographic range of the taxon, the larval food, and the data of the material examined. In addition, the descriptions are often accompanied by some general explanatory remarks pertaining to relationships, nomenclature, and other pertinent information. Also included in the paper is a very extensive selected bibliography covering eight and one-half pages. The study terminates with 29 plates of precisely delineated comparative illustrations of the larvae, drawn mostly by the author.

Jerome G. Rozen, Jr.

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CONTENTS OF FIRST ISSUE

BROOKS, M. A. Growth-retarding effect of carbon-dioxide anaesthesia on the German cockroach.

CASE, J. F. The median nerves and cockroach spiracular function.

EVANS, D. R. and DETHIER, V. G. The regulation of taste thresholds for sugars in the blowfly.

HASKELL, P. T. The influence of flight noise on behaviour in the desert locust (*Schistocerca gregaria* Forsk.).

KOIDSUMI, S. Antifungal action of cuticular lipids in insects.

LEGAY, J. M. Etude des modifications de certaines propriétés des oeufs de *Bombyx mori* L. dans plusieurs générations successives selon l'alimentation.

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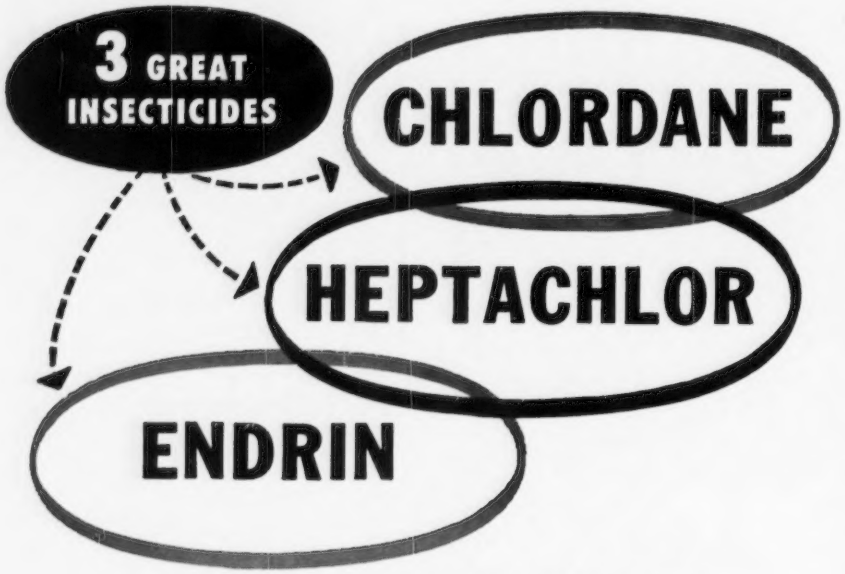

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TABLE OF CONTENTS

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The Physiology of Insect Cuticle.....V. B. Wigglesworth
Some Aspects of Intermediary Metabolism in Insects.....M. Rockstein
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Transmission of Disease Agents by Phlebotomine Sandflies.....S. Adler and O. Theodor
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TABLE OF CONTENTS

PAGE

MOORE, THOMAS E., AND HERBERT H. ROSS—The Illinois Species of *Macrosteles*, with an Evolutionary Outline of the Genus (Hemiptera, Cicadellidae) 109

ROSS, HERBERT H., AND THOMAS E. MOORE—New Species in the *Empoasca fabae* Complex (Hemiptera, Cicadellidae) 118

BROOKS, MARION A.—Succinoxidase in the American Cockroach 122

HAMMEN, CARL S.—A Growth-Promoting Effect of Cholesterol in the Diet of Larvae of the House Fly, *Musca domestica* L. 125

LUDWIG, DANIEL, AND MARY C. BARSA—Effects of Added Substrates and Inhibitors on Homogenate Respiration During the Metamorphosis of the Mealworm, *Tenebrio molitor* Linnaeus 128

PRESSER, BRUCE D., AND CHARLES W. RUTSCHKY—The Embryonic Development of the Corn Earworm, *Heliothis zea* (Boddie) (Lepidoptera, Phalaenidae) 133

DEAN, H. A.—Predators of *Oligonychus pratensis* (Banks), Tetranychidae 164

BECK, STANLEY D., AND J. F. STAUFFER—The European Corn Borer, *Pyrausta nubilalis* (Hübn.) and Its Principal Host Plant. III. Toxic Factors Influencing Larval Establishment 166

STONE, ALAN—Notes on Types of Mosquitoes in the Hungarian National Museum (Diptera, Culicidae) 171

BRELAND, OSMOND P.—Variations in the Larvae of *Culex stigmatosoma* Dyar with Notes on Similar Species (Diptera: Culicidae) 175

BELKIN, JOHN N., AND WILLIAM A. MCDONALD—A New Species of *Aedes* (*Ochlerotatus*) from Tree Holes in Southern Arizona and a Discussion of the *varipalpus* Complex (Diptera: Culicidae) 179

GALLARDO, E., A. J. FORGASH AND F. R. BEAUDETTE—An Exploratory Investigation of the Effect *in Vivo* and *in Vitro* of Cockroach Tissues on the Viability of Newcastle Disease Virus 192

FRICK, KENNETH E.—Nomenclatural Changes and Type Designations of Some New World Agromyzidae (Diptera) 198

ALLRED, DORALD M.—The Male, Deutonymph and Protonymph of the Mite *Eubrachylaelaps circularis* (Ewing) (Acarina: Laelaptidae) with Notes on Morphological Variations 206

Book Notices 178, 210

ERRATUM 174